

Planning for next year's summit

Last weekend's London summit was not pointless. And the working group on high technology should seize the opportunities for building a rational framework for innovation.

THE conventional wisdom is that last week's meeting of the heads of government of the seven principal industrialized nations accomplished nothing, which is inevitable. If 4,000 journalists are sent from all over the world to write about a meeting whose proceedings take place in private, frustration and boredom will ensure that no other conclusion will be reached. The nine previous summit meetings were in this respect no different from last week's in London. If those involved are concerned that they are so persistently misunderstood, they have one simple remedy — to hold their private meetings privately, without saying in advance where or when. Then, people's expectations would not be magnified to the point at which they can only be disappointed.

On this occasion, for what it is worth, three substantial benefits have accrued in the eyes of those began by expecting nothing, the chief of which is what the meeting had to say about the management of international debt. The issue is neither arcane nor academic. Increasingly during the past two years, it has become plain that the economic health of all the industrialized nations would be seriously if temporarily damaged if a substantial part of the international monetary system should collapse under the present strain. But why should it matter to people in comfortable industrialized countries, whose chief interest may be the pursuit of some interesting line of enquiry in research, that some distant government should be bankrupt? Surely only a few commercial banks will suffer? That is only half the truth. The Western commercial banks, profit-making and privately held enterprises though they may be, are an essential part of the economic system. And they fall like dominoes, as the events of the 1930s showed all too clearly. The default of a government on its loans, or the collapse of part of the banking system for that reason or some other, would be tantamount to a deflationary impulse within the economy of the industrialized West that would certainly snuff out the fitful economic recovery and might well be worse. The pursuit of research objectives of all kinds would be impeded.

Gloom

Last week's summit acknowledged the truth of this gloomy outlook, which is a good sign. The final communique goes one step further, and says explicitly that the seven major governments will use their influence with their commercial banks to help other governments that "are themselves making successful efforts to improve their position" to pay their debts, chiefly by giving them more time to pay. This is a step forward, but a small step. Better would have been a firm declaration on the size of the United States deficit, which is a cause of high international interest rates because it is not matched by private domestic saving. (Japanese governments can safely run huge deficits because thrifty Japanese, saving for their retirement, forgo current consumption to help out their government.) The statement earlier this week by the new government of Argentina that it had not so far agreed with the International Monetary Fund on a programme of austerity, which is how the summit governments would have it "take steps" to improve its position, suggests in any case that what is now proposed will not work. Politically, the industrialized nations cannot let a newly elected government in Latin America go to the wall, but the commercial banking system could not absorb the debts which it has piled up. On this crucial issue, the summit accomplished something, but probably too little.

On East-West relations — and arms control particularly — the summit declaration is more encouraging. What seems to have been agreed is that the seven members differ in their eagerness to resume negotiations of some kind with the Soviet Union — and have been given the assurance of the others that, up to a point, to do so will not count as rank disloyalty. The result, inevitably, is that governments other than that of the United States will be trying their luck in Moscow in three months ahead. President François Mitterrand, indeed, announced immediately after the summit meeting that he will be off late in the month. By coincidence, the Pugwash organization came to the conclusion, at its Geneva meeting at the beginning of the month, that this was precisely a period when explorations of what might be done on the limitation of nuclear arms would be more productive than formal negotiations. Part of the explanation is that the United States will soon be in the thick of an election campaign. But that, by itself, is no reason why President Reagan should not bring forward the Threshold Test-Ban Treaty for ratification (see *Nature* 7 June, p.482).

Innovation

The third respect in which last week's meeting deserves at least lukewarm applause is that a gathering concerned chiefly with the world's economy found time to approve, if only formally, a report from the Working Group on Technology, Growth and Employment (see p.572). The credit for that goes to President Mitterrand who, in the days of M. Pierre Chevènement, then the French minister for research, startled the Versailles summit two years ago with his passionate advocacy of technological innovation as the engine of the modern economic system — and as the best way of solving more immediate problems. And while the habit of listening politely to what the working group has to say may be a pale shadow of the Mitterrand evangelism of two years ago, by accident the summit members seem to have created the essence of an institution that could yet render invaluable service to them and the rest of us. Much will depend on what happens in the months ahead.

The working group, although a creature of the member governments, is mercifully above the political battles in which most of them are habitually engaged. No doubt that will remain the case so long as the group is not drawn into arguments about the rights and wrongs of monetarist as distinct from Keynesian economics, or other contentious matters of that kind. Its first instincts, exercised in the preparations for last year's summit at Williamsburg, were to encourage member governments to take the lead in collaborative technical projects (eighteen altogether).

Gingerly, it has now gone so far as to refer to some of the ways in which the governments which are collectively its master make things needlessly difficult for themselves by their imposition of restraints on technology. This year's topics are the restraints on the free flow of information ordained by strategic considerations (chiefly in the United States) and the tendency of all governments to believe that they too can excel at everything provided they deny themselves and their industries the benefits of technologies developed elsewhere, sometimes by tariffs, sometimes by other means. While the working group's report does little more than define these problems, it has in the process won for itself a chance to contribute to their solution. ►



This is why it is not too soon to begin preparing the group's report for next year's summit. The most urgent need is that governments should be made to understand the benefits of genuinely free trade in technology, a principle too often illegally denied even within the framework of organizations such as the European Community. Directly or indirectly, all the governments represented in London last weekend are in the business of supporting technological innovation, always by some device for making sure that public purchasing of high-technology products should benefit domestic producers. The immediate consequence is always to postpone a true division of labour in high-technology products, but in the long run, as water finds its own level, it is discovered that it is best, and most economical, for people to manufacture only those parts of products which they can make most economically. Meanwhile, for as long as this truth can be concealed, everybody loses.

The psychological trick that the working group should aim to engineer in preparation for next year's summit is that of persuading member governments that not all of them can be the world's sole supplier of, say, telecommunications equipment or of biologically engineered pharmaceuticals, but that these (and other) technologies are promising enough to allow everybody a sizeable share of a huge cake. That task will not be accomplished by rhetoric alone, for which reason there is an urgent need that the working group's next report should be soundly based in careful analysis and research. In passing, the working group might earn itself some extra credit by securing some practical recommendations on how to save effort in high-energy physics or planetary exploration (see p.573) by sensible collaboration between governments. The group's existence may be fortuitous, but the opportunities in its grasp would not otherwise easily be created. Let us hope it seizes them. □

Postscripts on India

The letters on Indian science on p.578 are the first of a flood. They deserve a careful reading.

WHEN the issue of *Nature* concerned with the condition of science in India appeared on 12 April, the troubles in the Punjab had been rumbling away for at least a year. They have since, as the world knows, exploded at Amritsar, and everybody's concern is that the largest true democracy ever will survive intact. Indians are always rightly saying that many of their apparently built-in obstacles to efficiency and effectiveness derive from their seemingly perverse attachment to democratic ways. The view that the maintenance of that condition of civility is more important than efficient public administration cannot easily be denied, although it is remarkable and courageous that India, with all its social problems, should have kept to that ideal.

The comments which now appear on the survey of the condition of Indian science (and earlier correspondence on the subject) should be read against this background. Some of the more obvious features of the Indian way of running academic and scientific institutions, open access at the universities for example, are largely consequences of the attachment to democracy. (And there is no reason why a state with more than 700 million people should not have more than 140 universities.) But when times are peaceful, there are good reasons why attention should more seriously be paid to the principles of good administration in these institutions. Laboratory directors and vice-chancellors need more freedom to direct their institutions; their colleagues and their students need more tangible goals on which to pin their ambitions. The huge scientific community could itself do much to improve the standards to which the majority work — and there are signs that such a movement is under way.

The question why India needs a scientific enterprise on such a scale, when there is all that poverty (and now Amritsar) needs only the simplest of answers. It has paid off, and handsomely. What is called the Green Revolution is a direct consequence of the investment there has been in India in agricultural research over the past few decades. □

Solar System unknown

A modest case for collaborative exploration of the Solar System deserves cautious support.

THE intended planetary explorers of the United States and Europe are well on the way to breaking records for advance planning (see p.573). Their proposals for a series of collaborative projects would, if accepted, put a cap on planetary exploration between now and the end of the century, at least so far as the industrialized West is concerned. (The Soviet Union and Japan, and perhaps other entrants to this expensive way of gathering data about the Solar System, may yet have surprises up their sleeves.) By comparison, even those who build accelerators are fleet of foot, counting themselves unlucky if they spend more than six years in building a new, more powerful machine. The reason why planetary projects are so long in gestation is that each of them is based on a purpose-built space vehicle packed with an array of instruments, most of which must be specially designed and developed afresh for each intended mission. Costs are also high — \$500 million seems to be the standard planning unit. So the question inevitably arises whether there is not some simpler and cheaper way of gathering the same kinds of data. Precisely that question has prompted the US National Aeronautics and Space Administration (NASA) to design a series of modular spacecraft for future planetary exploration.

Within the terms of reference of the joint US-European study, the report now published is nevertheless as sensible as could be expected. Its hallmark is indeed its modesty. One of the assumptions is that the programmes of planetary exploration planned separately by NASA and by the European Space Agency (ESA) will continue, and that the objective of collaboration between the two continents should be to mount projects that are unlikely otherwise to be carried out, yet which together do not carry spending on either side of the Atlantic above the limits likely to be found tolerable. Plaintively, the report says at one point that it would be easy to add to the list of explorations now proposed (to Saturn, six asteroids and Mars) if only there were funds available. The sad truth is that there is unlikely to be unforeseen growth in the budgets, and that the US commitment to the development of a space station (noncommittally endorsed by last week's summit meeting) is likely to take up any slack that may emerge. For the time being, at least, planetary exploration will be a poor cousin to the other projects in the two agencies' portfolios.

While the cost of these space missions is necessarily high, the more serious difficulty for those who would see more spending in this field is conceptual. Once a decision has been made to launch a spacecraft towards, say, Saturn, it becomes impossible not to pack the vehicle with a whole array of instruments devised to glean information about several unrelated phenomena. The result is that these enterprises take on the character of expeditions to distant places of the kind mounted by terrestrial explorers in the nineteenth century. Two of the projects now suggested (to the asteroids and to Mars) have the further disadvantage that they are meant as spurs to the development of new technology (solar-electric propulsion and robotized exploration), further clouding the objectives. It would, however, be a shame if European planetologists have to twiddle their thumbs for the next decade and a half, brooding over the meagre harvest of data ESA plans to provide. If the worst comes to the worst, at least the Saturn project should be mounted.

Meanwhile, planetary explorers should bend their minds to two other tasks, one technical and one conceptual. The case for planetary exploration would carry more conviction if space probes were simpler, cheaper but also faster — having to plan now to make observations more than a decade hence is to be bound to an inflexible chariot-wheel. And it would also strengthen the planetologists' case if they were to list the questions about the Solar System that need most urgently to be answered and then to specify the data that most directly bear on them. Part of the trouble now is that the questions they are asking are not pointed enough to justify spending in lumps of \$500 million. □

UK higher education

Cheers but no extra funds for engineering

THE British Government's ambition to redirect higher education towards engineering and physical science has been at least temporarily set back. At a meeting last week (4 June) called by Sir Keith Joseph, Secretary of State for Education and Science, it became plain that other spending ministries were not prepared to contribute by a reduction of their own budgets towards the cost of what the government is proposing.

There is particular resentment among officials of the Department of Education and Science that the Department of Trade and Industry, the chief agitator within the government for an increased output of graduates in engineering and physical science from British universities and polytechnics, was in the event unwilling to back its rhetoric by an agreement that funds should be diverted from its own budget for a new investment in higher education.

Last week, Sir Keith Joseph was asking for an extra £400 million to be spent over the next several years in universities and polytechnics, chiefly on new buildings and equipment for engineering and physical science departments. The government appears to have been convinced that such a development is urgently necessary by the well publicized complaints by engineering companies that they are having to recruit high-technology specialists from overseas and by evidence from within the educational system that the provision of buildings and equipment has for several years fallen short of needs.

In Whitehall, the plan to redirect resources towards engineering and physical science education has for several months been known as "The Switch", in retrospect a galling echo of the title of the 1970s film about confidence trickery, "The Sting". The objective has been to engineer a decisive and, with luck, permanent shift in British higher education towards the preparation of students for work in high-technology industry. The estimated cost of £400 million is predicated on the assumption that it would be better to strengthen and enlarge departments which are already strong (and fully stretched) than to take up unused capacity at other institutions.

Even so, one of the surprises of last week's meeting (attended both by Mr Norman Tebbit, Secretary of State at the Department of Trade and Industry, and by Mr Nicholas Ridley, Financial Secretary to the Treasury, the man who administers public spending) is that the estimated cost of £400 million seems not to have been questioned. It is not at this stage clear whether the extra investment in engineering education would have been

accompanied by an increase of student numbers at British universities.

While the meeting seems unanimously to have denied Sir Keith contributions towards his £400 million, the spending ministries represented at the meeting seem to have been less niggardly with their promise of wholehearted support for what he is trying to accomplish. But his direct request to the Treasury for the extra funds seems to have been met with the familiar statement that "further study" would be necessary.

With time unexpectedly on their hands, Sir Keith and Mr Tebbit appear to have fallen into a desultory and even nostalgic conversation about the advantages there

would be if only UK students were able to finance their own residence at universities. Schemes for replacing government maintenance grants by student loans, fashionable at the beginning of the present government's spell of office, have long since been abandoned because they offer no immediate relief to the public treasury.

The chance that the Treasury may find part of the funds needed to engineer The Switch in time for the financial year beginning in April 1985 is nevertheless not identical with zero. But many educationists are relieved that the plan has for the time being been derailed, if only because British higher education institutions are still in the thick of adjusting to straitened budgets — and bracing themselves for the probable erosion of the boundary between universities and polytechnics. Even so, in the aftermath of Sir Keith Joseph's failure with his begging-bowl, the University Grants Committee is likely to be devising a system of carrots and sticks to persuade universities towards engineering education. □

The year 2000

Prophets of doom rejected

Washington

A BLISTERING riposte to the pessimistic forecasts contained in the Carter Administration's 1980 *Global 2000 Report to the President*, was unveiled in Washington last week by the Heritage Foundation. *The Resourceful Earth*, a new book coedited by the late Herman Kahn and a Heritage Foundation economist, Julian Simon, says the *Global 2000* study is full of factual errors and illogical and misleading arguments. The book says the *Global 2000* warning that by the year 2000 the world will be more crowded, more polluted and less stable ecologically is "dead wrong". Its own happy prognosis is that the world in 2000 will be less crowded (though more populated), less polluted and more stable ecologically.

The Resourceful Earth concedes that the Earth's population will grow by the year 2000, but argues that better housing and mobility will make life less crowded for most people. In the richer countries, it says, there is evidence that hazardous air pollution has been declining and water quality improving. In rich and poor countries alike, life expectancy is increasing and average income has risen. Acid rain, the book says, is troubling, but could be mitigated by a greater reliance on nuclear energy. And the prospect of running out of energy, given the availability of nuclear power, is "purely a bogeyman".

The body of *The Resourceful Earth* consists of a score of papers examining trends in population, agriculture, climatic trends, energy, health and the environment. According to Simon, many of the authors were incensed by the "bad science" of *Global 2000* and were determined to put the record straight. Although almost all the

papers are robustly optimistic, the introduction (which appears under the names of Simon and Kahn although Kahn apparently died before he was able to make a significant contribution), warns against complacency. It concedes that all is not well everywhere and that a better future will not happen automatically or without effort.

Why the stark differences between *Global 2000* and *The Resourceful Earth*? Mainly, says Simon, because the report to the President failed to use enough trend data and, when it did, used them badly. Thus *Global 2000*, analysing data up to 1975, predicted continuing stagnation in the size of the fish catch. *The Resourceful Earth* claims that data for the years since 1975 show the fish catch resuming its long-run upward trend.

If there is one thread of pessimism in *The Resourceful Earth* it has to do with people rather than nature. Several papers complain that science and ingenuity offer ready solutions to many of our problems but humans and their institutions have a habit of botching them. A persistent example offered in the book is the reluctance of Americans to embrace nuclear power. In a dissenting statement at the end of the book, one author, University of Pittsburgh physicist Bernard Cohen, says he cannot share the optimism of his fellow authors because science has come under "irrational attack by the forces of ignorance". Public fear of nuclear power, he argues, is the fault of the media, "a group of scientific illiterates drunk with power, heavily influenced by irrelevant political ideologies".

Peter David

The Resourceful Earth: A Response to Global 2000 will be published in July by Basil Blackwell.

British polygraphology

Getting into business

THE polygraph or lie detector has arrived in Britain. A recently established company, Polygraph Security Services Ltd, now offers polygraph tests as a means of combating employee theft, and boasts that in at least one case now pending, charges of theft have been brought after "identification" of a suspect with the polygraph. In another instance, a man who failed a polygraph test was later found to have previous undeclared convictions for theft and was consequently dismissed.

Directors of Polygraph Security Services recently appeared before the House of Commons Select Committee on Employment, which is conducting an inquiry into the implications of the polygraph for industrial relations. The company declined to identify its clients to the Commons committee, citing commercial confidentiality. The committee accordingly threatened to use its legal powers to obtain such information, and the company's directors are now taking legal advice.

The chairman of Polygraph Security Services is Sir George Terry, a former chief constable of Sussex police. The other directors are Mr Jeremy Barrett, Mr Philip Tite and Mr Martin Seligson. Tite and Seligson are directors of Triangle PD Ltd, a printing and publicity company, and Seligson is also a director of Beneficial Arts Ltd, a company which distributes films of horse races to social clubs for fund-raising events. Barrett is director of a market consultancy company. None of the directors has previous experience of polygraph tests. Their training has been through the Zonn Corporation in the United States, of which Polygraph Security Services describes itself as the United Kingdom extension.

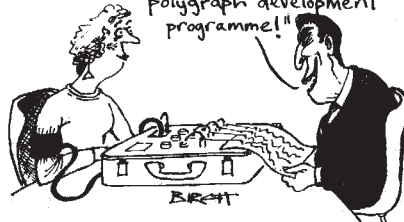
Seligson claims that with the polygraph, companies can cut employee theft by 80 per cent. Some client companies are already conducting tests on job applicants, and an existing workforce can be screened regularly through the "honesty maintenance programme" offered by Polygraph Security Services. Seligson stresses that all tests are voluntary, although it is unclear how such a screening programme could possibly be effective if a substantial proportion of the target group declines to be tested.

Seligson is unimpressed by the serious questions that have been raised about the reliability and efficacy of the polygraph. He says that Professor David Lykken, who wrote a critical article on polygraphic interrogation published in *Nature* in February (307, 681; 1984) is "totally debunked" and "generally discredited". The authority for this judgement is a paper by D.C. Raskin and J.A. Podlesny published in the *Psychological Bulletin* in 1979 (86, 54-59). The empirical issues separating those authors from Lykken boil down to what

sort of data is valid for an objective test of the polygraph's effectiveness. Lykken charges that Raskin relies on studies using pre-selected polygraph traces which had previously been scored "correctly" by a polygraph examiner, so providing evidence only that trained polygraphers tend to concur in their judgements.

UK polygraphers are not above circulating (non-attributably) rumours to the effect that Lykken administered or undertook to administer a polygraph test to Mr John de Lorean, now on trial in California, on behalf of de Lorean's defence lawyers. Lykken's version of this story is that the judge in de Lorean's case threw out all polygraph evidence after seeing two directly contradictory polygraph reports: one, from the Federal

"Congratulations! You've failed—you're a compulsive liar—utterly dishonest—just the person we need for our polygraph development programme!"



Bureau of Investigation, concluded that the accused was deceptive, while the other by Raskin concluded that de Lorean was truthful with 99 per cent probability. Lykken denies ever giving polygraph tests himself except as demonstrations, and says his total involvement with the case was one telephone call.

While Polygraph Security Services is already advertising its services in Britain, little is known about plans to introduce polygraph testing for those employees at the Government Communications Headquarters in Cheltenham who have access to highly classified material. The British Security Commission recommended last year, after a review of the Prime spy scandal, that polygraph testing should be considered for such employees. It is thought that about 20 senior Cheltenham personnel volunteered to take part in a pre-test of the polygraph in London, although some others may have declined. There are no longer any formal links between the majority of employees at the communications headquarters and the civil service unions, but there are not thought to be any immediate plans to extend the test. But, according to one account, it has already been decided in principle to make polygraph tests mandatory for all employees at Cheltenham during their probationary period, and for those with access to highly classified material at each quinquennial security review.

Tim Beardsley

Summit science

Leaders espouse collaboration

THE London summit last week gave an uncontentious blessing to a report of the Working Group on Technology, Growth and Employment — the gathering of senior government science advisers which owes its existence to President François Mitterrand's initiative at the Versailles summit two years ago. But the secret of the report's easy passage seems to have been that its references to contentious issues were cast in the most anodyne language.

On the perennial bone of contention between the United States and the six other summit countries — the control of Western technology by means of the strategic embargo — the report says only that strategic considerations have persuaded governments to seek to control technology and that, "nevertheless", the exchange of information and the encouragement of trade are necessary for the maintenance of technological innovation.

Dr Robin Nicholson, Chief Scientific Adviser at the UK Cabinet Office and *ex officio* chairman of the working group during this calendar year, explained earlier this week that the immediate objective had been to win the acknowledgement of governments that strategically inspired restraints on the free flow of information and products were a potential impediment to the pace of technological innovation. He said that the issue may be explored in greater detail at the two sessions of the group due to take place before the next summit, next year in West Germany.

Other impediments to innovation such as "non-tariff" barriers to international trade are also referred to in the report, which acknowledges that these often stem from the "wish of countries" to be self-sufficient in high technology. Nicholson is especially keen that the working group should encourage uniform and acceptable standards for the new technologies.

The working group's chief initiative since the Williamsburg summit a year ago seems to have been to urge the need for international cooperation on environmental problems, especially acid rain, radioactive waste disposal, the marine environment, the greenhouse effect and development of power sources free of potentially harmful emissions. The working group rejected the notion that these questions should be added to the list of eighteen projects mounted by the time of Williamsburg. Now the summit proper has asked for a detailed report and action plan by the end of this year.

The projects already begun under the auspices of the summit working group are a mixed bag, ranging from thermonuclear fusion, biotechnology and robotics to an attempt to explain people's resistance to new technology (in which the United

Kingdom volunteered to take the lead). According to Nicholson, the robotics projects, led jointly by France and Japan, were a particularly rich source of collaborative research projects, not merely in production technology but in mining, firefighting and agriculture.

The emergence of the working group as an institutional part of preparations for the annual summits of the industrialized nations is something of a surprise. Nicholson explained this week that its objective is largely to identify areas where collaborative research may be useful, whereupon it will be for individual countries to take the lead in fostering particular collaborative projects among summit members. On his view, some of the areas thus identified are certain to be more fruitful than others, while everybody seems

reconciled to the notion that some projects will fail for lack of interest.

Even so, Nicholson concedes, there may be a need that the working group should monitor the fate of the projects which its recommendations stimulate. As things are, proprietary information arising in the course of collaborative research projects would remain the property of the partner paying for the research concerned, while information about the conduct of research projects would not necessarily be passed to all interested parties. During the past year, however, it seems to have been agreed that membership of these partnerships should be extended to countries not among the seven summit-going states, with the proviso that outside membership should be allowed when the interests of the project would thereby be served. □

Planetary exploration

Plans for US-Europe launches

A WORKING party of European and US planetary scientists has made a strong bid for a modest layer of frosting on the cake now promised by their respective agencies, the European Space Agency (ESA) and the US National Aeronautics and Space Administration (NASA). The three projects, originally made public at a meeting organized by the working party at Heidelberg in West Germany at the end of April, were put to a meeting of the Royal Society in London last month. But the working group's hope of explicit backing by last weekend's economic summit has not been borne out.

The essence of the recommendations from the joint working group, led by Eugene H. Levy from the University of Arizona and Hugo Fechtig from the Max-Planck-Institut für Kernphysik at Heidelberg, is a set of three planetary missions designed to be complementary to the programmes supported separately by ESA and NASA, and taking account both of Soviet plans (especially for Halley's comet and on Venus) and those being developed in Japan. The projects are:

- A spacecraft bound for orbit about Saturn but releasing *en route* a probe destined to crash on the surface of Titan, the largest satellite of Saturn, measuring atmospheric and aerosol constituents of the atmosphere of Titan on the way. The object would be launched in 1992.
- Exploration of up to half a dozen asteroids would be attempted by means of a spacecraft directed successively to chosen asteroids, spending about a month in orbit about each of them in turn. The feasibility of this project hangs on the development of a solar-electric propulsion system. Recommended launch date is 1994.
- The most ambitious project is a plan to put an automatic vehicle on the surface of Mars for the collection of geochemical and geological data. Both the landing craft and the vehicle would present considerable

technical challenges to those working on the project, for which no launch date has been set except that it should be before the end of the century.

The projects singled out by the working group for priority were originally chosen so as to provide opportunities for a measure of independent collaboration in which respective US and European halves would be developed and built independently. The working group says that the first two projects could be contained within the budgets now foreseen for planetary exploration by NASA (\$300 million a year) and by ESA, but that the third, perhaps the joker in the pack, would require that extra funds should be set aside.

The working group report emphasized that the three projects are intended to be complementary to the existing planetary programmes of the two agencies. NASA's future strategy is now tied to the development of a series of modular low-cost spacecraft that would be launched from the shuttle by a Centaur rocket. ESA's programme depends on the use of Ariane. The working group also draws attention to opportunities for closer coordination between the existing plans of the two agencies, the coordinated launching of the US and European Martian satellites due to be launched in 1990.

The working group was established jointly by the European Science Foundation and the Space Sciences Board of the US National Academy of Sciences, each of which has endorsed the recommendations. The test of its success will, however, lie with the responses of the operating agencies, NASA and ESA. The former plans in the next several years to concentrate on the development of low-cost planetary exploration as part of its Observer programme, while European planetologists grumble that they are poor cousins to the astronomers in ESA's forward planning. □

Indian nuclear power

Breeder not so fast

New Delhi

THE Indian Atomic Energy Commission has announced that a tiny reactor using uranium-233 as fuel has become critical at the Bhabha Atomic Research Centre (BARC) at Trombay. This is cheering news at a time when the nuclear power programme is under fire for time and cost overruns in commissioning nuclear reactors, frequent breakdowns and a meagre output of the necessary heavy water despite investment.

The reactor concerned is the only operational reactor in the world fuelled by uranium-233, a man-made fissile isotope of uranium produced by irradiation of thorium in a reactor. The fuel has been produced BARC using a research reactor and reprocessing plant.

For India, the importance of the development lies in its long-term nuclear power programme which is to be based on conversion of thorium to uranium-233. BARC has now shown that this may be feasible. The southern Indian states of Kerala and Orissa have deposits of thorium large enough to sustain atomic power generation for more than a hundred years. Eventually, reactors with uranium-233 fuel may be used as a basis for a fuel cycle which breeds new fissile material.

The tiny reactor Kamini developed at Trombay, uses about 500 grams of uranium-233, and will be commissioned at Kalpakkam research centre near Madras in the next three months. It is estimated that even a test reactor will have to be 100 times bigger than Kamini. Meanwhile, a 15 MW test breeder is nearing completion at Kalpakkam. Indian scientists have developed plutonium-uranium oxide fuel for this reactor. France was to have supplied the fuel, but the deal has fallen through because of what India calls the "prohibitive price" demanded by France.

India's second phase of atomic power development is based on the fast breeder system. Feasibility studies and a design are nearing completion for a 500 MW sodium-cooled reactor to be set up in the next decade. The reactors in the first stage are based on pressurized heavy water and indigenous natural uranium. Only the Tarapur plant is using the enriched uranium now being supplied by France.

India has set a target of 10,000 MW for its nuclear power capacity at the turn of the century, which would meet 10 per cent of the expected total power requirements. Five projects are already in operation, with a nominal capacity of 2,240 MW, and two more under construction will add another 510 MW. But, with a gestation period of as much as 10 years, there are doubts about whether India will meet the ambitious 10,000 MW target.

Sunil Saraf

US agricultural research

Congress digs in heels

Washington

HOPES for an expanded programme of competitive grants for agriculture have been dashed once again by Representative Jamie Whitten (Democrat, Mississippi), chairman of the powerful House of Representatives Appropriations Committee. Last week, the House went along with Whitten's wishes, approving major cuts that his committee had made in the Reagan Administration's request of \$50 million for the programme. The new initiative in biotechnology under the programme was cut from \$28.5 million to \$10 million. Plant science was cut to half of its present level of \$15 million (also the amount requested for next year). Also missing from this year's \$34,000 million agriculture budget is \$5 million approved last year for graduate fellowships.

Representative George Brown, who has been fighting year after year to increase support for competitive grants above the \$17 million level where it has stagnated since its inception in 1978, offered an amendment on the floor of the House to restore \$10 million of the cuts to biotechnology. The amendment was rejected on a voice vote. According to Brown's staff, there was little hope that the amendment would be passed ("no-one bucks the chairman"), but Brown believed he had made his point and may have garnered some quiet support that will help when the bill goes to House-Senate conference. The Senate, which has yet to act, is likely to agree with the full administration request.

Supporters of an expanded programme of competitive grants had been optimistic this year. Many longstanding opponents — notably the land-grant colleges, which had tended to view competitive grants as a threat to their assured non-peer-reviewed federal support — have recently changed sides. The association of land-grant colleges produced a report last autumn recommending \$70 million for competitive grants for biotechnology and two US Department of Agriculture (USDA) research advisory panels had made similar recommendations. The President's science adviser, Dr George Keyworth, has also lent his personal backing.

During the floor debate, Whitten twice dismissed basic research as "finding answers for answers' sake" and suggested that biotechnology research was "based on hopes" that do not address farmers' immediate needs.

Besides ordering the cuts, Whitten's committee earmarked parts of the competitive grants funds for specific research projects — a development that the programme's supporters say threatens to subvert the principle of investigator-initiated peer-reviewed research. Most of the earmarking clearly reflects the committee's desire to rescue congressional

pet projects known as "special grants", scheduled to be phased out this year. USDA has never cared much for special grants, a programme of mandated research that gives the department little flexibility. The special grants programmes in soybean research, acid precipitation and alcohol fuels were transferred in their entirety to the competitive grants plant sciences section, a total of \$1.7 million in earmarked funds; two new sections were created in the competitive programme to accommodate \$3.5 million in special grants programme on aquaculture, gypsy moths, boll weevil and pine bark beetle; and, apparently for lack of a better place to put it, \$250,000 for "sugar cane in Hawaii" was inserted in the biotechnology section.

Data protection

UK bill improved and approved

THE British Government's Data Protection Bill, substantially modified during its committee and report stages, was finally approved by the House of Commons at its third reading last week. The bill sets out to provide for the first time the statutory right of access for individuals to data that are held about them in machine-readable form, while at the same time preventing unauthorized disclosure of such information. Despite the highly contentious exceptions to these general principles, the official opposition declared itself content with the concessions offered by the government last week and did not oppose the bill, which now returns to the House of Lords. It is expected to gain the Royal Assent within the next month.

One of the most controversial clauses of the bill would have allowed medical data to be passed to outside agencies such as the Inland Revenue or the police by people other than health professionals. A medical inter-professional working group under the chairmanship of Sir Douglas Black has drawn up a code of practice to govern the disclosure of medical information, and after extensive lobbying the government has now accepted that these should in principle apply to computerized health data wherever they occur. A government amendment to the bill will allow the Home Secretary to bring in codes of practice, based on those to be used within the Health Service, wherever these precautions are thought necessary.

Universities and examination boards had been worried that the bill's provisions would allow examination candidates access to their marks even before the examination results were officially published. That anomaly has also been rectified; candidates for examinations will in future be allowed access to their marks only 5 months after the request, or 40 days after the examina-

Taking into account the cuts and earmarking, the competitive programme received only an additional \$497,000 in unrestricted funds over the current appropriation.

Meanwhile, there are increasing signs that USDA's in-house research arm, the Agricultural Research Service (ARS), is taking biotechnology seriously. Both Beltsville, ARS's chief research centre, and an ARS laboratory in Albany, California, are to become centres for biotechnology. Beltsville officials are speaking of quadrupling research in this area over the coming years. The Albany laboratory will also receive increased support, and collaboration is planned with researchers at the University of California at Davis. Most of the new effort will apparently be supported by a shift of existing funding, since ARS is to receive only a 3 per cent increase in next year's budget.

Stephen Budiansky

tion results are published, whichever should be sooner. Some university vice-chancellors had, however, hoped that candidates' access would be delayed until 90 days after publication of results, to discourage frivolous challenges to the accuracy of examination data from disgruntled candidates.

Other modifications incorporated during the bill's committee and report stages will allow individuals access to data held about them for the purposes of immigration control, and will allow individuals damaged through inaccurate data the right to compensation for damage and distress. The data protection registrar is to play a more active role than was originally foreseen, promoting codes of practice among data users and pursuing complaints raised by individuals about whom data are held.

Blanket exemption in the bill's earlier version that would have allowed the Home Secretary to exclude any or all government-held data from the bill's disclosure provisions have been considerably curtailed, and small data banks held solely for payroll accounting purposes will not now have to register under the act.

Last week's debate was remarkable for the unaccustomed affability and cordiality displayed between the Home Office minister involved, Mr David Waddington, and the speakers of the official opposition, mainly Mr Robert Kilroy-Silk and Mr Denis Howell. Only Liberal Members of Parliament seemed still determined to score every point they could. It seems that the committee debates on this controversial bill were more than usually productive, and most of its former opponents seem satisfied with the changes that have been made. The bill will allow Britain to ratify the European Convention on Data Protection.

Tim Beardsley

NSF moves

Knapp replaced by Bloch

Washington

DR Edward Knapp, director of the National Science Foundation (NSF) for the past two years, will leave his post at the end of this year to return to Los Alamos. This was announced earlier this week by NSF, which said that Knapp will be succeeded by Erich Bloch, now an IBM vice-president, who has spent all his working life with the company.

Knapp's departure is unexpected. During his spell in Washington, he has secured for NSF some of the most substantial budget increases since the foundation's inception. Over the past two years, the budget has grown by more than 35 per cent while NSF's traditional involvement with science education, virtually eliminated during the early years of the Reagan Administration, has been partially restored.

Knapp has been an energetic and stout defender of the NSF's role in the support of

basic science but also a loyal member of the administration. One striking feature of his tenure of his office is that the posts of deputy director and of the three statutory assistant directors at NSF, vacant when he was appointed, have remained unfilled.

Bloch is by background an electrical engineer who, unlike his predecessors, has no doctorate. At IBM, he was responsible for the development of the micro-processors for the IBM 360 series of computers. Since 1968, he has held a number of senior management positions with the company and is at present in charge of technical personnel development.

Bloch was born in Germany in 1925, and became a US citizen in 1959. He is a member of the National Academy of Engineering. The director of NSF is appointed by the US President for a six-year term, which appointment is subject to confirmation by the Senate.

Stephen Budiansky

British nuclear power

BNF bites contamination bullet

BRITISH Nuclear Fuels PLC has announced that it is planning a £100 million study to find ways of reducing radioactive discharges into the Irish Sea to a level as near zero as possible. The decision was announced last week by Mr Con Allday, the company's chairman, who stressed that the new policy was in direct response to public concern about radioactive discharges from its reprocessing plant at Sellafield (formerly Windscale).

Declaring that the company must respond to "fears which are genuinely held, however ill-founded", Mr Allday said BNF would "not attempt to fight a rearguard action on behalf of rationality". The company would recognize the strength of public opinion and would take steps to reduce discharges to "very much lower levels than hitherto planned".

The company has been through a disastrous few months for public relations. Last November, a television documentary drew attention to a locally increased incidence of leukaemia in children near the reprocessing plant at Sellafield and raised the possibility of a link with the plant, which emits Europe's largest routine discharge of radioactivity into the environment. The documentary spurred an urgent inquiry, now in progress, by a team headed by Sir Douglas Black, a former president of the Royal College of Physicians, and formerly Chief Medical Adviser to the Department of Health.

Days later an unplanned discharge of radioactively contaminated chemical solvent led to public warnings from the government to avoid the use of beaches in the area, a warning recently withdrawn.

The company's conduct was later criticized in swingeing terms by the Department of the Environment. More recently, transport unions have threatened to block the delivery of spent fuel to the plant unless the levels of discharge are reduced.

Discharges of both beta-gamma emitters and alpha-emitting actinides from the plant are down to a tenth of the peak levels of the early 1970s. Sir Denys Wilkinson, until recently chairman of the government's Radioactive Waste Management Advisory Committee, said last week at a lecture organized by the Science Policy Research Unit of the University of Sussex that radiation dose to critical groups resulting from the Sellafield operations was "higher than one would like it to be". The original fluorescein experiments on which the discharge policy was based were planned and executed in 4 months, Sir Denys recalled. There had been no full evaluation of the consequences of the discharges, and unforeseen developments such as the lowering of plutonium safety limits and the "uncooperative attitude" of the Irish Sea in returning radioisotopes to land instead of dispersing them into the ocean had reduced margins of confidence. In addition, discharges were unnecessarily high in terms of what is easily achievable. Discharges from the French reprocessing plant at Marcoule, for example, were substantially less than that at Sellafield. Sir Denys was nevertheless convinced that nobody had suffered any significant extra hazard as a result of British waste disposal practices, and he expressed doubts as to whether Sellafield could achieve the low emissions reached at Marcoule.

IOS closes lab

THE UK Natural Environment Research Council (NERC) has decided that there is a *prima facie* case for closing the Taunton site of the Institute of Oceanographic Sciences (IOS), which carries out research in sedimentation and applied wave mechanics. The council's governing body was due this week to consider the implications of transferring the work to other NERC sites.

Staff at Taunton are unimpressed with the way NERC has handled the matter. The director of IOS, Dr Anthony Laughton, was said to have promised speedy decisions on the future of the site almost a year ago. Dr Laughton declined last week to make any comment about the proposed closure.

Staff at Taunton do not see where they can go. NERC's budgetary problems mean it cannot put up any new buildings, and there is not thought to be room for existing Taunton staff at the IOS site at Bidston on Merseyside. Most would prefer instead to move to the site of the Institute for Marine Environmental Research at Plymouth.

The accommodation problems will probably be solved by the resignation of many of the staff. Scientific manpower at Taunton is already much less than it was in the past; the decreased emphasis on wave power as a likely future renewable energy source may be part of the explanation. Finance from the Department of Energy has been repeatedly delayed, causing difficulties for those planning research. But, in theory at least, research should not be cut back as a consequence of the move now planned.

Tim Beardsley

The £100 million study now proposed by BNF is above and beyond current action to reduce discharges costing an estimated £150 million. An ion-exchange plant now nearing completion will cut down discharges of radiocaesium, and a separate salt evaporation plant will substantially cut actinide discharges. Further reductions may be brought about by improvements to the reprocessing plant itself.

The recent events at Sellafield have postponed any possibility of a public offering of shares in BNF, which are at present all held by the government. There have also been extensive changes made at senior management level.

The company is still awaiting the decision of the Director of Public Prosecutions on whether criminal proceedings should be started against BNF over November's debacle. Possible infringements of operating licences were afterwards reported by the Nuclear Installations Inspectorate.

Tim Beardsley

Correction

THE limit for nitrogen oxides in ambient air proposed in the EEC directive (*Nature* 308, 309; 1984) should have read 200 gm^{-3} not 200 mg m^{-3} . □

Mercury pollution

Dry battery alarm in Japan

Tokyo

A PAIR of headphones and a portable cassette player have become pretty much standard street wear for young Tokyoites, but they are creating a new problem for the Japanese Government — how to dispose safely of the batteries that power them. Alkaline-manganese batteries have been favoured because they provide a stable voltage and last a long time: the trouble is that they contain a lot of mercury which, it is feared, is finding its way into the environment from municipal rubbish tips.

The risk of mercury poisoning raises strong emotions in Japan. In 1956 and 1964, major outbreaks of Minamata disease, caused by eating shellfish contaminated by methyl mercury in factory effluent, left thousands of victims, some paralysed from birth.

Public protest over risks from batteries led this week to an announcement of subsidies and tax incentives for industry to set up a special non-profit research and development association to find ways to make mercury-free batteries. This move has, however, neither been welcomed by industry nor been seen as much more than a sop to public opinion by consumer groups. Industrial companies have been trying for years to take the mercury out of batteries — simply because it is so expensive — but without finding a suitable alternative.

Japan is now manufacturing a staggering 2,850 million dry batteries each year. About two thirds of these are for domestic use and the 70 tons of mercury they contain — mainly in the alkaline-manganese batteries and “button” type mercury batteries used in cameras — end up in domestic rubbish, most of which is used in landfill in 2,500 municipally owned sites. According to calculations at the National Institute of Environmental Science, the research arm of the environment agency, these sites will have an average battery-derived mercury content of about three parts per million — a thousand times higher than the maximum mercury content permitted in water supplies — on top of a small amount of mercury from discarded fluorescent lighting tubes and other sources. As the batteries corrode, the mercury will enter the environment and an unknown fraction of it will be converted into organic mercury by bacteria and leached away. Most sites are small and are converted to other uses within five years; being municipally owned, they are often converted to public use, including the building of schools and playgrounds. The risks are much less from the small fraction of rubbish that goes to incinerators rather than to landfill. Incinerator temperatures are far above the boiling point of mercury and the concentration of vapour reduced into the air is low — much lower than the World Health Organization guideline levels although still

thousands of times greater than natural background levels.

Consumer groups are now demanding that batteries be collected and disposed of separately from other rubbish. Already about 400 of the nation's 3,300 municipalities have passed local regulations requiring citizens to deposit their used batteries at special disposal centres rather than in household rubbish, and that number is expected to grow to 800 by the end of the year. All the local authorities can do with the batteries at the moment, however, is to store them until some safe disposal method has been found.

Radioactive waste

Mongolian alarm at China's plan

CHINESE plans to act as a “nuclear dustbin” for Western countries have provoked a sharp reaction in the neighbouring Mongolian People's Republic. Alarm was crystallized last month in a formal interview, organized by the Soviet news agency TASS and its Mongolian equivalent Montsame, with a Mongolian physicist, Dr D. Dorjbat, who appears to have been selected as a mouthpiece of grass-roots scientific concern.

According to Dorjbat, the Chinese do not possess a highly developed nuclear technology, and so will not be able to ensure a reliable method of storing the wastes. Since they plan to bury them beneath the Gobi Desert, there is a serious risk of contamination of Mongolian as well as Chinese territory, through seepage of radioactive material in the groundwaters.

Dorjbat went on to discuss the political implications of the plan. The Chinese, he alleged, not only hope to obtain additional hard currency from the plan (their preliminary tenders offer to take up to 4,000 tonnes of waste by the end of the century thus earning \$6,000 million); they also want to process the waste into plutonium for nuclear weapons. Numerous “foreign experts”, he said, have drawn the same conclusion.

Dorjbat made no attempt to explain how, at the same time, the technological level of the Chinese could be too low to deal with wastes but high enough to extract plutonium from those wastes. He seemed more at ease with his political statements, alleging that China is at present helping Pakistan to develop nuclear weapons (a claim which the Pakistanis vehemently deny). He also attacked the “disrespect” for the destiny and lives of the Chinese people shown by their leaders who, he claims, regard a nuclear war as winnable.

Beijing, so far, has made no direct reference to the allegations, but appears to be treating them as significant signals in the current war of words with Moscow. Within

Dr Tsukehiro Gotoo, chief researcher at the National Institute of Environmental Science, hopes to see the construction of five battery reprocessing plants across the country together with the enforcement of a refundable deposit system to encourage the return of used batteries. A private battery reprocessing plant is already in operation at Hokkaido but its charges are high — 80,000 yen (£260) per ton of batteries plus transport charges. Studies at the institute show, however, that setting up regional recycling plants with a capacity of 2,000 million batteries a year would at most add only Y10 (£0.3) to the cost of a battery. Such a move seems more likely to provide a practical solution to the problem than the call of the Ministry of International Trade and Industry (MITI) for research on non-mercury batteries.

Alun Anderson

a few days, *The People's Daily* carried what was clearly a direct refutation of the attacks on the waste disposal plan, affirming that Chinese technology was well-equipped to deal with all forms of nuclear waste, solid, liquid or gas. The more sinister charges that the Western wastes would be used as the raw materials for bombs was, however, pointedly ignored.

Vera Rich

Moscow visit off

Washington

AMID continuing uncertainty about the health of Soviet physicist Andrei Sakharov and his wife Yelena Bonner, the US National Academy of Sciences confirmed last week that its president had postponed plans to visit the Soviet Union to re-establish formal links with the Soviet Academy of Sciences.

In a short telegram to A.P. Aleksandrov, president of the Soviet Academy, Dr Frank Press, president of the US National Academy, said talks on a new agreement were “impossible” given the deepening concern of American scientists over the circumstances of Dr Sakharov. The telegram added: “I have delayed until the last moment a final decision in the hope that the situation would be positively resolved in a manner that all of us would recognize. Since this hoped-for development has not occurred, I believe it is to our mutual advantage to postpone the visit of our delegation until a climate more favourable for positive discussions exists.”

Although academy officials stressed that the decision to cancel the planned visit was taken by the academy alone, they acknowledged that Dr Press had consulted the government. The officials also stressed that the visit had been postponed rather than cancelled, and hinted that it could go ahead if the Soviet Academy showed signs of interceding on behalf of Sakharov and Bonner.

Peter David

Nuclear winter

ICSU project
hunts for data

AN urgent call for information on the consequences of large ground-based fires for the atmosphere of the Earth has gone out from the workshop held in Leningrad between 14 and 16 May on the climatic and biological consequences of nuclear war. The meeting is part of the programme by which the Scientific Committee on Problems of the Environment (SCOPE) of the International Committee of Scientific Unions (ICSU) proposes to produce a comprehensive report on the subject by September 1985.

Participants at the Leningrad meeting explain that one of the obdurate uncertainties of the prediction of sharply reduced and even sub-zero temperatures on the surface of the Earth after a nuclear war, called the nuclear winter, concerns the likely behaviour and effects of the large amounts of smoke from fires that would be carried into the atmosphere. The aggregation of smoke particles is, in particular, not taken into account in the models of the effect so far published.

At the Leningrad meeting, Soviet scientists suggested that data bearing on this question might be gathered from studies of large Siberian forest fires, to which end a meeting on the subject is to be convened in the Soviet Union, followed by another in West Germany.

The possibility was also canvassed of collecting data from the forest fire that has been raging during the past two years in Indonesia. One difficulty appears to be that the resolution of satellite data, both spatially and in time, may not be sufficient for the model builders. The SCOPE office responsible for the project thinks it would be valuable to have the services of an aircraft to make observations of major fires occurring in the next twelve months, but is not hopeful that it can find the resources.

On the design of climatic models, the Leningrad meeting emphasized the importance of full three-dimensional models of the general circulation taking account of the "scavenging" of particles from the atmosphere. One of the particular difficulties to be surmounted, according to a statement after the Leningrad meeting, is that of accommodating mesoscale (intermediate scale) atmospheric turbulence in the general circulation models.

The reactions of participants at the Leningrad meeting to the general question whether nuclear winter is a probable consequence of a major nuclear exchange appear to be mixed. Some stress the need for more data and better model studies. One Western participant said, however, that he had been impressed that "the nuclear winter is becoming more real all the time". Some claimed to have detected a difference between what they called the Moscow

school (represented at Leningrad by V.V. Aleksandrov), and the Leningrad school (K. Ya. Kondrat'ev), with the former being more certain than the latter that a nuclear winter would follow a nuclear war.

The next workshop within the SCOPE project will be held in Paris in October. Drafting of the final report is due to begin at a meeting in Colorado next January, but the final version of the report will be com-

pleted only at a meeting of the project team arranged for next June at the University of Essex in the United Kingdom.

Part of the problem that lies ahead is financial. Most of the support for the SCOPE project, launched at the Cambridge meeting of ICSU two years ago, consists of a grant of \$75,000 from the Rockefeller Brothers' Trust, but other possible sources are being approached. □

AIDS development

NIH to license HTLV

Washington

THE companies selected by the US federal government to manufacture a blood test for acquired immune deficiency syndrome (AIDS) are expected to be announced this week. A scientific panel has already met to review the 20 or so proposals received in response to an announcement in the *Federal Register* on 3 May, and recommendations have been forwarded to Assistant Secretary for Health Edward Brandt.

The selected companies will be provided with Dr Robert Gallo's method for mass-producing human T-cell leukaemia virus III (HTLV-III) in return for a royalty payment to the government that is expected to be 5 per cent. The *Federal Register* announcement specified that applicants should have experience with retroviruses, virus production and the manufacture and distribution of radioimmunoassay kits, and have access to a P-3 containment facility. Although skill in recombinant DNA techniques was also mentioned, members of the scientific panel that reviewed the applications said they gave far greater emphasis to ability to manufacture the product quickly and in large quantity. Millions of test kits will be required each

year to screen blood products. It is expected that the initial production will rely exclusively upon existing technology — growing the viruses in bulk to produce enzyme-linked immunoabsorbent antibody (ELISA) tests to detect the virus. This widely used technique provides a sensitive and simple means of assaying serum antigens. Eventually, recombinant DNA techniques may be used to produce virus antigen for the tests.

Abbot, DuPont and Roche are said to have submitted strong proposals. More than one company is expected to be chosen.

Genentech has submitted a proposal through its joint venture with Travenol, Genentech-Travenol Diagnostics. Genentech is also known to be seeking a basic research collaboration with Gallo and perhaps also with the French group at the Pasteur Institute. Gallo is said to be in something of a dilemma over the possibility of collaborating directly with a private company; on the one hand he needs the expertise in gene expression that the private companies have, but he is also concerned about giving preference to one company outside the context of the licensing agreement. **Stephen Budiansky**

Nature index of biotechnology stocks

12-Month high	12-Month low	Company	Close previous month	Close 31 May	Change
14	8	Biogen (Switzerland)	11 3/4	9	-2 3/4
2	1 3/8	Bio-Logicals (Canada)	1 3/4	1 1/2	-1/2
14 3/8	8	Bio-Response (USA)	13 1/8	8 7/8	-4 1/4
14	10	Cetus (USA)	12 1/4	10 1/4	-2
10 3/8	5	Collaborative Research (USA)	7	5 3/8	-1 5/8
19 7/8	11 1/2	Damon (USA)	15 7/8	11 7/8	-4
26 1/4	12 1/4	Enzo-Biochem (USA)	16	14	-2
10 1/8	5 1/4	Flow General (USA)	7	6 1/8	-7/8
42 1/4	28 3/4	Genentech (USA)	35 1/2	29 1/2	-6
10 3/4	5 3/8	Genetic Systems (USA)	6 5/8	5 3/4	-7/8
17 1/4	8 1/4	Genex (USA)	12 1/2	9 1/8	-3 3/8
23	12 1/4	Hybritech (USA)	17 1/2	12 1/8	-4 3/4
16 1/4	8 3/4	Molecular Genetics (USA)	11 1/2	9 1/2	-2
15 1/2	10	Monoclonal Antibodies (USA)	14	10 1/4	-3 3/4
60 7/8	43	Novo Industri A/S (Denmark)	51 3/8	43 1/2	-8 1/8
22 3/4	14 1/2	Pharmacia (Sweden)	17 1/8	14 1/2	-2 5/8

Closing prices are for the last Friday of the month. For over-the-counter stocks, bid price is quoted; for stocks on the American and New York exchanges, the transaction price. *Nature's* weighted index of biotechnology stocks stood at 135 on 31 May, compared with 164 a month earlier. Data from E.F. Hutton, Inc.

Science in India

The supplement on Indian science Nature 12 April (pp. 581-600) has prompted many reactions. Some are published here, with more next week.

SIR — The key to considering science and technology in India lies in recognizing that India is trying to go through three revolutions simultaneously — the agricultural (still incomplete), industrial and computer revolutions — with a government that is democratic, a population less than half of which is literate and resources that are hardly adequate for a task never before undertaken. This is the reason for the wide spectrum from poverty to excellence that one finds in India in all aspects of life. I do not think that *Nature's* supplement recognized this, explicitly at any rate.

A few careless statements crept in — for instance, "India... often describes itself as the third nation in the world of science and technology". The truth is that India does state frequently that it has the third largest scientific manpower, but mainly to illustrate that despite that fact, productivity is low in science, industry and everywhere.

But my main objection is to the way you depicted the central universities as "breeding grounds for violence", apparently on the basis of the report on the working of the central universities. The report has nothing to do with the actual work of the central universities and much less with science in them; rather it is concerned with factors preventing the central universities from working as they ought to. The report is in no way a guide to what the central universities are, any more than a pathologist's report reveals a man's personality.

In fact, the original concern of the report was precisely that of "finding some way to restore authority to where it belongs". For several decades, long before the Indian Institutes of Technology started to blaze their trails, the central universities, notably those of Delhi, Banaras and Aligarh, contributed much to science and humanities. It is totally wrong to say "nepotism, disorder (even violence) and maladministration are rife even in the seven (central) universities...", wherever this information came from.

Your sampling of the scientific institutions and scientists also appears to be inadequate. Unless one is careful, the visible and the vociferous have a high probability of looming large. But surprisingly, after all that is said, you have not given much, except, perhaps, a few more counts in the *Science Citation Index*. Yes, ambivalence seems to be a part of our relations.

But despite these complaints, and although I do not always agree with your conclusions, your survey is the most incisive analysis of the complex system of science in India. I agree with you that "India has by far the best chance of succeeding" in the development of science

and technology, not because of "the ingenuity and articulateness of its people", but because, as Aldous Huxley pointed out (*Science and Literature*), one of the greatest achievements of science is to have developed a method independent of those who work with it.

B. S. RAMAKRISHNA
*University of Hyderabad,
Hyderabad 500 134, India*

SIR — You are, of course, right in saying that all is not well in India's Science. But great strides have been made. As our Prime Minister declared during a White House reception, India is engaged in the stupendous task of moving hundreds of millions of people into the twentieth century. Against this backdrop one can understand why our laboratories and classrooms are no longer sheltered. The attendant erosion of academic freedom, loss of objectivity and so on are inevitable, but, one expects, temporary phenomena.

Notwithstanding the problems, there have been some achievements (as noted by you also). The green revolution is a reality, and our chemical industry is able to lean on our own research and development. Where high technology is concerned, we can certainly claim we know how to launch a satellite and how to build a nuclear power station. On the more exotic basic research side, we cannot boast of a string of spectacular discoveries, but then our total investment relative to that in the West is paltry. If achievement is measured relative to obstacles overcome, our record measures up favourably to those of others.

All the same, we are neither satisfied nor complacent. Recently our two academies held a joint brainstorming session to examine how to improve matters. It was discovered (as expected) that streamlining Indian science was about as easy as holding a plasma at a million degrees in a container at laboratory temperature, and for essentially the same sort of reason. But some suggestions did emerge and they have been transmitted to the Science Advisory Committee to the Cabinet.

We are confident we will make it and are working hard towards that objective. As you remark, "the doubt is not whether but when". Meanwhile, our expatriate friends could note that Indian science cannot be bettered by simply asking "Is this all we have done...?" (Incidentally, what have those giving this advice done for us?) Nor will it be accomplished by listening to seminars by scientists coming for a family reunion or even under the auspices of an international committee.

G. VENKATARAMAN
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SIR — Readers of your survey are likely to conclude that most of what is good and worthy of its name in Indian science is so because (1) a director or a chairman or perhaps a minister knows and means his/her business; (2) they or their organizations have or at least had good overseas connections and (3) they have somehow managed to avoid the anything-but-beautiful influence of the run-of-the-mill scientific workers and of the people at large.

The major contradiction in Indian science is that of its patch excellence *vis-à-vis* its unrootedness in Indian soil. Co-operation from scientifically advanced countries is necessary and should be welcomed. India needs to ensure a certain degree of immunity against the crisis, of which there are visible signs, by directing science in international directions. But the country also needs help in promoting the search for identity of the "two Indias".

With the general elections expected this year, the political undertone of the report carries significance. However "surprising" and "dangerous" it may be for Indian science to be strongly influenced by the people in power, in fact its infective power seems to defy territorial confinement.

Lastly, for the sake of correctness of information, the Bose Institute of Calcutta is not and never was engaged in training graduates, nor is there any emphasis on nuclear physics in its research programmes. Neither of the two Boses received Nobel prizes, on which point there seems to be some misunderstanding (p.589-90).

R. MAJUMDAR
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SIR — Your special issue on India provides a comprehensive account of accomplishments, failures and problems of science in India. The introductory note to the survey, however, contains the erroneous figure of 2.5 per cent as the annual rate of India's current population growth. The registration of births and deaths in India is far from complete, but the annual population growth rate of 2.2 per cent during 1971-81, derived from the census counts of 1971 and 1981, is considered by demographers as reasonably accurate. In a recent statement, Prime Minister Indira Gandhi gave the current annual growth rate as 1.9 per cent. The figure of 2.5 per cent instead of 1.9 per cent in the article does not, of course, affect its content or quality, but for India it means an increase of 19 million people every year instead of 14 million. Rapid population growth is a serious problem that Indian scientists and policymakers must tackle.

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Genetics and heritable IQ

A new study of IQ among adopted siblings points to hereditary intelligence, but inconclusively. Geneticists (and others) should not however be fearful of a determination of the question.

Is human intelligence genetically determined, environmentally conditioned or the product of both influences? Since the work of the late Cyril Burt was discredited some years ago, the ferocity seems to have gone out of the argument on this question that had been raging for close on half a century. The article that appears on p.620 of this issue, yet another piece of evidence that is by itself inconclusive, will in no sense reawaken that argument. It may however be an appropriate occasion for asking what would happen if ever the question were finally decided.

What T.W. Teasdale and David R. Owen have done is to attempt to discover in measurements made with genetic siblings brought up separately, usually as a consequence of adoption, the degree to which intelligence (as represented by measured IQ) is genetically determined. The study has been made possible by the accessibility of the adoption register in Denmark, and by the ready availability of data on educational attainment, IQ and height (as well as other physical attributes) for males who must register for military service at the age of eighteen. The availability of these data is in part a justification of the pleas that social scientists have been making in recent years that legislation to protect the privacy of individuals should not disallow access for the purposes of legitimate research.

The numbers involved in the study now reported are comparatively small. Five groups of sibling pairs (all male, since women do not register for military service) have been identified, of which two groups have been used, for practical purposes, as controls. One of these groups comprises genetically unrelated males adopted by the same parents and brought up together, the other a larger group of genetic brothers brought up by their natural parents. One essential element in the design of the study is the use of measurements of height (at the age of eighteen) as a kind of control variable. There is a wealth of earlier evidence to suggest that height is indeed heritable, so that height and IQ should behave similarly if, indeed, IQ is inherited.

The outcome of the study, so far as it goes, favours the hypothesis that intelligence is indeed genetically inherited. This, at least, is the outcome of a correlation between the IQs of adopted siblings reared apart. The standard calculation that this result may have arisen by chance yields a satisfactorily low value — the odds are one in 200. But as with other

studies of this kind, only some of the crucial questions that will arise in discussion of these data have at this stage been taken up. How comparable were the families into which the several groups of children were adopted? May there not be systematic differences between the families adopting related and unrelated siblings (other than the observation by Teasdale and Owen that parents adopting more than one child are likely to be well off)? One odd feature of the data now reported, however, is that the expected correlation in height among separately reared siblings does not unambiguously show up. The explanation may be the small size of the groups of adopted children, but until other explanations are excluded, the precise significance of this study cannot be certain.

None of this implies that the study has no immediate bearing on the question of whether intelligence is heritable. On the contrary, and at the very least, the study is an imaginative pointer to a source of data on heritability that has previously been almost entirely overlooked. One especially valuable feature of the Danish data is that the records of adoption and the measurements of IQ have been carried out for quite different purposes than that of the authors. Even if Burt's studies of genetic inheritance in identical twins had not been discredited by the suspicion that they may have been crudely fabricated, the fact that he was personally responsible for the measurement of IQ would by now have put his claims in doubt. One of the immediate consequences of what Teasdale and Owen have done will be to stimulate a search for other objectively gathered blocks of data that may throw light on the heritability of intelligence.

But why bother? One common assertion is that when a positive result (in favour of heritability) would be socially divisive, no good purpose can be served by answering the question. Such a response cannot however be regarded as a serious impediment to serious enquiry. If indeed IQ were shown unambiguously to be genetically determined, the result would be interesting in its own right and stimulating of other investigations. And even if it were the case that IQ is only a crude representation of what should properly be understood by intelligence, it would be even more remarkable that such an artefact, constructed from the ease with which individuals carry out artificial tasks, should be genetically determined than that unmeasured and per-

haps unmeasurable intelligence should thus be determined.

Precisely what the practical consequences would be of a conclusive determination of the heritability of IQ is another question. In the ancient row between the nature and nurture schools, it has commonly been assumed that the truth must be uncomfortable for one side or the other. That view should carry very little weight. For nobody pretends that if IQ is inherited, the effects can be as simple as in, for example, the inheritance of well-known physical disorders where a single gene may be responsible.

Everybody agrees that IQ, if inheritable at all, is determined by several genes, perhaps even indirectly. The result is that the IQ of the offspring of parents of known intelligence will be determined by some distribution spanning a range of measured values. Offspring of high-IQ parents will frequently have low IQs. Exactly the same kind of overlap is found in the distribution of IQ in racially different groups of children attending the same schools.

The consequence of these expectations of how measured IQ is likely to be distributed is that even when the question of heritability has been settled, few useful guides to social policy will have been provided. It will remain the case, for example, that societies that insist on educating the offspring of successful parents separately from others, on the twin assumptions that success denotes intelligence and that all offspring of intelligent parents are themselves intelligent, will imperfectly satisfy the needs of many of the children. It would be similarly profligate of human potential to suppose that, if IQ is heritable, schooling as such might be abolished on the grounds that native wit will make itself apparent.

In the whole field of social policy, exactly similar considerations apply. A determination of the question whether IQ is heritable would be largely irrelevant to policy, which is not to say that it would never be used as a means of justifying irrational prejudices. This is why studies such as those of Teasdale and Owen are warmly to be welcomed, why there is the strongest case for hoping that the issue will soon be clarified — and then for learning something of the mechanism by which such a fuzzy attribute of the central nervous system as intelligence may be, even in part, heritable.

John Maddox

Cancer chemotherapy

DNA amplification and multidrug resistance

from Piet Borst

SINCE 1978, when Robert Schimke and his co-workers showed that it could be associated with the development of drug resistance in eukaryotic cells¹, gene amplification has been shown to underlie resistance to several drugs and toxic compounds in a variety of eukaryotic cells, including such unicellular organisms as fungi and protozoa (see ref. 2 for an excellent recent review). There have also been hints that it underlies another form of drug resistance in mammalian cells — the intriguing multidrug resistance (or pleiotropic drug resistance) in which the induction of resistance to one drug leads to cross-resistance to a motley assembly of other drugs with widely different chemical structures and cellular targets²⁻⁶. Direct evidence that multidrug resistance is due to gene amplification has been missing. That lacuna is filled by the paper of Roninson *et al.* on page 626 of this issue⁷.

Schimke's studies were on the development of resistance to methotrexate (MTX), an inhibitor of the enzyme dihydrofolate reductase (DHFR). Resistance to it can arise in at least three ways^{3,4}: by a decrease in the carrier-mediated uptake of MTX; by the production of an altered DHFR; and by the increased production of normal DHFR. Schimke and co-workers showed that the third route involves an amplification of the structural gene for DHFR¹.

How does the genome know that DHFR has been blocked by MTX? The answer seems to be simple: MTX inhibits cellular DNA synthesis; when the block is released by removal of the drug, chromosome segments that have partially replicated will not only continue the old replication round but also re-replicate, producing an extra copy of that region. Once gene amplification is underway, selection with MTX ensures that cells with the largest number of copies of the *DHFR* gene will have the best chance of surviving. By stepwise increase of the drug concentration, one can eventually obtain cells with many copies of the gene. (For more on the relationship between amplification and replication the reader is referred to David Denhardt's article in these columns last week⁸.)

The extra gene copies produced by amplification may either be re-inserted back into chromosomes to yield homogeneously staining regions (HSRs) or remain as extra 'minute' chromosomes, often found in pairs as double minutes. HSRs are stable when cells are grown without drug, but maintenance of double minutes usually requires continuing drug selection because they lack a centromere. Random distribution of double minutes

among daughter cells and loss to the cytoplasm rapidly leads to the emergence of cells that have lost most or all of their double minutes; this instability is a hallmark of drug resistance due to this type of gene amplification (see refs 2, 9-11).

The presence of double minutes in multidrug-resistant cells, as well as the unstable nature of multidrug resistance, has strongly indicated that this, too, is due to gene amplification^{12,13}. Resistance of this type can be induced with colchicine, a drug that prevents tubulin polymerization, or with one of the carcinostatic anthracyclines, drugs with diverse effects on cells. Cross-resistance develops to a group of drugs whose only features in common are a molecular weight between 350 and 1,250 and an amphiphilic aromatic nature. This indicates that multidrug resistance is due to altered drug transport, but whether the mutation results in decreased drug entry, increased efflux or a combination of these mechanisms is still not certain²⁻⁵.

A corollary of this type of drug resistance is the overproduction of a large membrane glycoprotein with a molecular weight around 170,000. A reasonable hypothesis is that this membrane glycoprotein is encoded in the amplified DNA¹³. This cannot be the whole story, however, because different multidrug-resistant cell lines show different degrees of cross-resistance to individual drugs.

The direct evidence for gene amplification in multidrug resistance provided by Roninson *et al.* in this issue of *Nature* comes from the use of an elegant, in-gel, renaturation technique. The authors show that two multidrug-resistant Chinese hamster cell lines contain amplified DNA segments, absent in revertants. They have cloned one of the amplified segments as recombinant DNA in *Escherichia coli* and show that the degree of drug resistance correlates with the number of copies of this segment in the DNA of resistant cells. The two cell lines have different patterns of cross-resistance, and it is therefore of interest that the two lines have only part of the amplified DNA segments in common. This suggests that at least three proteins can contribute to multidrug resistance: one preferentially induced by anthracyclines, one by colchicine and one induced both by anthracyclines and by colchicine. It should not be too long before the gene products of these amplified genes have been identified.

What is the relevance of this sophisticated molecular biology to the clinician treating cancer patients? Only limited information is available to answer this question.

Amplification of the gene for DHFR has been shown to occur in tumour cells from patients who have had prolonged treatment with MTX¹⁴⁻¹⁸. Multidrug resistance is frequently observed in patients, but whether gene amplification contributes to it is not known. Once the amplified genes in multidrug-resistant human cell lines have been cloned it should be easier to test for amplification of these genes in tumour biopsies by standard molecular and cytological hybridization techniques. I expect that these experiments will give positive results in a significant fraction of patients exposed to intensive treatment with carcinostatic drugs. Most carcinostatic drugs block DNA replication; some of these drugs act on DNA in a similar fashion to certain carcinogens which are among the most effective inducers of gene amplification^{1, 19-21}; and the schedules of drug administration used by clinicians are not incompatible with induction of gene amplification.

If gene amplification is important for clinical multidrug resistance, a more complete understanding of the phenomenon might be useful. Knowledge of which gene is amplified could allow prediction of cross-resistance to other drugs. Knowledge of the detailed mechanism of drug exclusion could lead to the design of modified drugs not affected by the exclusion mechanism. The membrane proteins overproduced could form useful targets for antibody-coupled toxins. Multidrug resistance is obviously an area where gene amplification will induce research amplification. □

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Mathematics

Soap bubbles and catastrophes

from Ian Stewart

MANY mathematicians think in pictures, and maintain that geometric ideas offer some of the most important insights in the subject. Others, who think in symbols, disagree. Be that as it may, a number of topics in the mainstream of current mathematical research have clear links to geometry. A recent example¹ combines ideas from the theory of minimal surfaces with Thom's theory of catastrophes²⁻⁴ to shed new light on an old problem posed by soap bubbles.

The study of minimal surfaces goes back to the (blind) Belgian experimentalist Joseph Plateau, who in 1873 published a book, *Statique Experimentale et Théorique des Liquides soumis aux seules Formes Moleculaires*, dealing with the shapes assumed by soap films under various constraints. In particular he noticed that if a closed loop of wire is dipped in a solution of soap and glycerine, a surface forms, spanning the wire. Many elegant shapes can be constructed in this way. If gravity is ignored, such a surface will minimize the energy due to surface tension, that is, its area. Hence the name 'minimal surface'. The 'plateau problem'⁵ asks for a rigorous formulation of this concept, and a proof that an arbitrary loop can be spanned by a minimal surface. It has been solved, in various settings (for example, refs 6-8); it has also been considerably generalized.

Catastrophe theory, on the other hand, deals with discontinuous changes in the state of a system having an underlying continuous structure. In its simplest and best known version, elementary catastrophe theory, it studies systems that minimize some energy-like potential function. The best known example, the cusp catastrophe, provides a universal description of the local competition between two stable states. Despite criticism of some speculative models, catastrophe theory has proved applicable to a variety of problems^{9,10}, though not always those originally envisaged by its creators. Now another area of application has emerged to augment the ever-growing list, and to confound further the pessimists.

About a decade ago, Poston observed that soap films appear to undergo catastrophe-like changes when the loop that they span is slowly deformed¹¹. Specifically, if a loop is constructed in roughly the shape of the seam on a tennis-ball, the film will span two stable minimal surfaces (by symmetry); but if it is opened out into a flat circle, it spans a unique surface. Poston argued persuasively that the transitions between these competing states should be described by cusp catastrophe geometry, but gave no proofs. This conjecture has now been demonstrated rigorously by Beeson and Tromba¹.

They find it convenient to work with a specific shape of loop, yielding a surface known classically as 'Enneper's surface'. The variational nature of the problem (minimizing area) is highly reminiscent of catastrophe theory (minimizing potential), but there are technical difficulties. In particular, catastrophe theory is usually formulated for a finite-dimensional set of states, whereas minimal surface problems require an infinite-dimensional set. Nevertheless, Tromba has developed techniques^{12,13} to render catastrophe theory applicable to the minimal surface problem, although a number of obstacles must still be overcome. The results add to the available knowledge on a difficult and important problem: how many minimal surfaces are there that span a given loop? Indeed,

they furnish the first explicit example of a non-unique solution. The results also contribute to the generalization of catastrophe theory to variational problems in infinite dimensions, thereby bringing several new areas of application within its scope. □

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Botany

St Helena Ebony tree saved

from Mark Williamson

ST HELENA is probably best known as the oceanic island to which Napoleon was exiled. To biologists it is also known for its extinct flightless birds and remarkable endemic trees. It is also a classic example of the destruction of the biota of an oceanic island by man¹; the introduction of goats and other species and the stripping of bark for tanning have all contributed to the decline of the native flora. Therefore, particularly as 1984 is Plant Year of the World Wildlife Fund, it is a pleasure to record that about 12 species endemic to St Helena are now being cultivated², including one that had been thought to be extinct for 130 years.

The island is a mere 121 km², lying in the South Atlantic at 16° south. It is ringed by cliffs rising to 200 m or more, while the centre of the island rises to just over 800 m. It was formed by volcanic action 7-15 Myr ago, in the Miocene. A mere 36 species of flowering plants are definitely native^{3,4} and no fewer than 33 of these are endemic. Another 20 or so species may be native, but even with them the list would be remarkably short. An equivalent area in England would have roughly ten times as many species.

Two of the most remarkable of the endemic species are the Redwood⁵ and the Ebony, now placed in a genus of their own, *Trachetiopsis*⁶, in the Sterculiaceae, a chiefly tropical family which includes the Cocoa and Kola trees. The nearest relatives of the Ebony and Redwood are the six species of *Trochetia* found only on the islands of Mauritius and Réunion in the Indian Ocean. The Ebony seems to have been a dominant small tree in the lower arid

parts of the island, while the Redwood was found in the more mesic zone higher up⁷.

Whereas the Redwood has continued to survive on St Helena, albeit now only in gardens, the general assumption had been that the Ebony became extinct sometime after the 1850s. But in November 1980, George Benjamin, a forest guard, and Quentin Cronk, of the University of Cambridge, found two bushes of Ebony growing on a cliff⁸. In January and February 1983 Cronk was back on the island with Simon Goodenough, of the Royal Botanic Gardens, Kew. Cuttings from the Ebony were established^{2,9} and the plants have now flowered at Kew. The Ebony can be propagated both by cuttings and by seeds, and there are now plans to plant several hundred back on the island². Since all the plants are derived from two bushy individuals, the genetic type and range of the survivors may differ appreciably from the generality of the original stock. But it looks as though the species has been saved. □

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Rheumatoid arthritis

Another viral candidate

from K.A. Brown

DEVOTEES of the school that believes an occult virus is responsible for the induction of rheumatoid arthritis will have welcomed a recent article summarizing a three-year collaborative study undertaken in three USA centres on the identification of parvovirus-like particles in synovial tissue from the joints of rheumatoid arthritis patients¹. For the sceptics in this field, the publication heralds the arrival of yet another viral candidate whose association with rheumatoid arthritis, if any, will be shown to be circumstantial and which warrants investigation simply because it is one of the few viruses which has not already been the subject of an attempt to associate it with the disease.

The research of Simpson *et al.* stems from their isolation of an infectious agent, termed RA-1, from rheumatoid synovial cells, co-cultured with human lung fibroblasts or rabbit fetal synovial cells, and subsequently passaged in suckling mice. Injection of RA-1 into the cerebral area of the brain of neonatal Swiss-Webster mice produced a fatal neurological disease, whilst its inoculation into the peritoneum produced several symptoms, notably permanent crippling, alopecia, conjunctivitis and dwarfism. Such fatalities and disabling effects were not induced by synovial extracts prepared from patients with the non-inflammatory disorder osteoarthritis.

In their size (24–25 nm), morphology and resistance to chemical and physical treatment, the infectious particles resemble a parvovirus. Parvoviruses are among the smallest of the DNA viruses. There are two main genera: the adeno-associated, whose replication is 'defective' and requires the help of a co-infected adenovirus; and the adeno-independent, whose replication does not require assistance. In domestic animals parvoviruses are believed to be responsible for such conditions as panleukopenia and enteritis in cats and hepatitis in dogs and geese. Human 'serum parvovirus-like virus' was discovered during the screening of symptom-free blood donors for hepatitis B virus antigen². This adeno-independent virus, often referred to as the B19 virus, was shown to induce febrile illnesses in haematologically normal people³, aplastic crisis in patients with haemolytic anaemia due to infection of a haematopoietic progenitor cell⁴ and to be transmitted to haemophiliacs in blood clotting factor concentrates⁵.

Since established laboratory cell lines may be contaminated with parvovirus, there is always a risk of parvovirus contamination during the co-culture and passage stages of experiments. However, Simpson *et al.* show that injection of

rheumatoid arthritis synovial cells into murine brains, without prior co-cultivation with accessory cells, induces the appearance of the RA-1 virus. Furthermore, the RA-1 antigen does not react with antisera directed against several other parvoviruses which infect mice and other animals.

On caesium chloride equilibrium gradients, the RA-1 viral particles are found at a density of 1.30–1.43 g cm⁻¹. In a collaborative study which is shortly to be submitted for publication, Gabi Stierle, a visiting scientist to our department, demonstrates that caesium chloride fractions of 1.41 g cm⁻¹, prepared from synovial cells of five of eleven rheumatoid arthritis patients, but none of six osteoarthritis patients, react with an antibody against RA-1. The immunoreactive rheumatoid fractions contain abundant particles of 10 nm diameter whilst 23 nm particles are seen in similar isolates from long-term rheumatoid synovial cultures. The rheumatoid fractions do not react with the monoclonal antibodies to the B19 virus. This finding, together with the failure of the B19 virus to react with the anti-RA-1 antibody and the high prevalence of the anti-B19 antibody in 61 per cent of the population⁶, suggests that it is unlikely that the B19 virus has an important role in rheumatoid arthritis¹.

Parvovirus is the infectious agent of Aleutian disease in mink, a slowly progressive fatal disorder characterized by hypergammaglobulinaemia, anaemia, immune complex-mediated arteritis and

glomerulonephritis⁷ and thought by some investigators to have features of autoimmune disease. There is no evidence to suggest that parvovirus may directly initiate a synovitis, though the affinity of the virus for host cells that are of high mitotic activity, particularly activated lymphocytes⁸ and endothelial cells⁹, and its dissemination by monocytes and macrophages⁸ are attributes which might be expected of a polyarthritogenic agent. It is premature to indulge in conjecturing possible implications of the work of Simpson and Stierle for rheumatoid arthritis. We must await definitive characterization of the RA-1 virus by techniques such as cell hybridization, serological evidence of parvovirus infection, laboratory studies performed on patients with inflammatory arthritides other than rheumatoid arthritis, and cytopathogenetic studies using adenovirus and other viruses which might support its replication.

Simpson *et al.* are to be encouraged in their persistent and patient search for a replicating virus in rheumatoid arthritis, a task that seems fraught with almost insurmountable biological and technical obstacles. Whatever the outcome, they will have resurrected interest in an area which urgently requires more motivated scientists of enthusiastic application and original approach. Progress is painfully overdue. □

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100 years ago

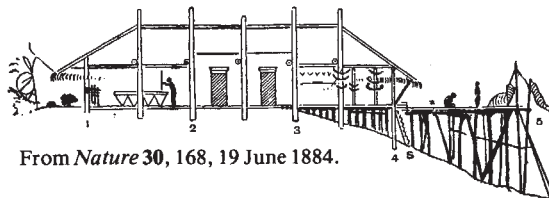
PILE-DWELLINGS ON HILLTOPS

AMONG the Miris, Singphus, Kamtis, Nogas, Mismis, and Deodhains that I have questioned as to the origin of the custom of building on piles, the answer is invariably that they do not know, that it is their tribal custom, &c. Pressed as to the advantages of it, or why they could not build on the ground like Hindus, they generally end by urging the absolute necessity of keeping things out of reach of the ever-present pig.

As an illustration of how this animal practically affects the question of house-building, I append a section of a typical average Noga house, as built by the tribes south-east of Sibsagar to the Upper Dihing. One end generally rests on the ground, while the other overhangs a slope for which there often seems to be no

occasion, as plenty of level land is about. In all houses of this type the end devoted to husking rice rests on the ground, and the door at the end has a slab that can be raised to admit pigs to eat the husks. This compartment (one-third of the house) is divided from the living and sleeping part by a wall and a door with a stile to keep out pigs. There are generally from two to six, or more, sleeping-rooms on the ground, and beyond them again is an open room used for visitors, or to sit and work in during wet weather.

But there are many other things besides pile-dwellings that prove these now distinct tribes to have descended from a common stock. The "morongs," or houses in which the lads and single men sleep at night, away from their parents' houses, are seen under various names all through these hills. There are also "morongs" for the girls and single young women, and there are special and peculiar laws relating to morongs. Liberty of the sexes before marriage is indeed practically so complete among all these tribes that really *morals begin with marriage*. After marriage they are better, I think, than civilised nations.



From *Nature* **30**, 168, 19 June 1884.

Catalysis

Zeolites under control

from Lovat V.C. Rees

ON page 589 of this issue of *Nature*, Haag *et al.* report a very nice example of the way in which zeolites can be used in the controlled catalysis of hydrocarbon cracking, which is best seen in the context of John Dwyer's brief but comprehensive overview of zeolites as acid catalysts in the 2 April issue of *Chemistry and Industry*.

Zeolites are crystals of aluminosilicates permeated by channels and cages of molecular dimensions. The active catalytic centres are hydroxyl groups — one for each Al atom — which are readily generated by deammoniation of ammonium-exchanged zeolites on heating to 400°C. On heating to temperatures greater than 550°C, however, these Brønsted acid sites are converted to Lewis sites by loss of H₂O. John Dwyer clearly indicates the importance of zeolites in elucidating the fundamental mechanisms involved in the catalytic conversion of hydrocarbons. Since zeolites can readily be synthesized over a very wide range of Si/Al ratios, it is possible to create catalysts with active centres whose average separation distance can be varied in a controlled way. Also, by choosing specific zeolite structures from a large list, channel and cage geometry can be varied. Specific channel geometries can also be modified by various techniques.

With this ability to finely tune zeolite catalysts, it is possible to observe the effect of increasing the acid strength on, for example, the product ratios resulting from the isomerization of butenes. The shape-selective behaviour of zeolite catalysts can be readily quantified. Thus reactant and product shape-selectivity and restricted transition-state-selectivity can be explained. The size and shape of the channels and cages exert both geometric and diffusional restrictions. The size of the crystals, coking, steam treatment and chemical modifications affect the products of a reaction in easily explained ways.

It is usually quite difficult to identify and count the number of active sites involved in a catalytic reaction but that is not the case for ZSM-5, the zeolite studied by Haag *et al.* and in which it is possible to have such a high ratio of SiO₂ to Al₂O₃ that the Al atoms are no longer a structure-determining constituent. Haag *et al.* varied the Al content of ZSM-5 from 20 to 10,000 p.p.m. such that the average distance between Al sites represented several to a very large number of atomic diameters and the distribution of the sites will have approached one of randomness. There is, thus, a substantial chemical uniformity to the catalytic sites.

The activity of these samples towards the cracking of *n*-hexane in controlled conditions was linearly related to the Al

content and extrapolated to zero activity at zero Al content. This quantitative correlation can be explained only if all Al atoms are in tetrahedral framework locations — which is exactly how they are shown to be arranged by high-resolution magic-angle spinning nuclear magnetic resonance.

With such accurate quantitative data it is possible to determine the number of active sites, the absolute molecular turnover rates and the turnover numbers. Haag *et al.* found an absolute turnover rate for *n*-hexane cracking of 2.8 molecules per minute per atom of Al over the entire Al concentration range. Since the reaction rate is first order, the fraction of occupied Al sites at 100 torr is less than 1 per cent of the total Al sites and the turnover rate per occupied active site (the turnover number) is greater than 300 molecules per minute per active Al site.

The reaction rates for other reactions, for example 1-hexane cracking and 1-hexene double-bond isomerization, have also been shown to be linearly dependent on the Al concentration. The olefin cracking rate is some 800 times the paraffin cracking rate while the olefin isomerization rate is some 1,300 times the olefin cracking rate. The Al concentration needed to achieve technically relevant reaction rates can be less than 1,000 p.p.m. for paraffin conversion and from a fraction of a p.p.m. to 100 p.p.m. for other conversions. Turnover numbers as large as 4×10^7 can be obtained with zeolite catalysts; not dis-

similar to the values in enzyme catalysis.

Ultra-stable zeolite Y is currently used as the cracking catalyst in oil refining. It cannot be synthesized with a ratio of SiO₂ to Al₂O₃ greater than 3, but this ratio can be increased by various de-alumination processes. These impart additional thermal stability but lower the crystallinity, and the resulting catalyst is still too active and requires dilution with catalytically inactive binder material. Even then, coking of the catalyst pellets occurs in a reactor in a time scale measured in seconds. The data of Haag *et al.* on ZSM-5 indicate how little Al is necessary for cracking reactions and should spur zeolite chemists on to discover a method to produce zeolite Y with very much less Al than used at present. Very interesting technical problems will have to be overcome to produce such a catalyst.

Finally, Martens *et al.* show that a relatively simple test can be used to obtain a detailed insight into the structure of the channels and cages of zeolites (*Zeolites* 4, 98; 1984). Using *n*-decane isomerization and hydrocracking reactions over platinum-loaded hydrogen zeolites as a fingerprint, five independent criteria can be found which give consistent and supplementary evidence on the structure and dimensions of the void space in zeolites. Voids that are characterized in terms of unidimensional pores made of 10-membered rings of oxygen atoms are readily distinguished from those that are cages of varied dimensions accessible through pores made of 8- or 12-membered rings. Surely the consistent reproducibility of these catalytic reactions indicates the uniqueness of zeolites as catalysts. □

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Immunology

T-cell development in the thymus

from Ken Shortman

MUCH of the development of T lymphocytes takes place in the thymus, away from the melee in bone marrow where B lymphocytes and other haematopoietic cells differentiate side by side. What is the purpose of this convenient packaging? Is the thymus an inductive environment for switching the differentiation of uncommitted stem cells into one of the T-cell lineages? Or is it an immunological finishing school, generating a diversity in the antigen receptors on the surface of T cells and selecting those most suitable for recognizing foreign antigens presented in the context of 'self' (major histocompatibility) antigens on the surface of cells. Or is it both? A careful study of what goes in and what comes out can set the limits of what might happen in the thymus itself.

Recently devised techniques^{1,2} for labelling cells within the adult mouse thymus and

for tracing labelled emigrants into the periphery have shown that emigrant cells are much like mature T cells, are already committed to one or other of the specialized T sublineages and are already functional. The essential development of the cells must have occurred within the thymus, or even before their arrival there. The output from the thymus is low, however: only about 3 per cent of the cells produced daily leave the thymus, the rest apparently dying within it. Although this high attrition rate suggests a stringent culling for appropriate specificity, it could equally well reflect a low efficiency in the formation of a functional T cell of any specificity.

The other part of the equation, the seeding of T cells in the thymus, is considered by Ezine, Weissman and Rouse on page 629 of this issue of *Nature*³. They have studied the reconstitution of the T-cell

population of the thymus of mice whose haematopoietic stem cells, including those for T cells, have been destroyed by irradiation. For the purposes of reconstitution, Ezine *et al.* supplied the mice with a mixture of a large number of bone-marrow cells from identical mice and a small number from congenic mice that carried a different T-cell surface marker. It was thereby possible in immunohistological studies of frozen thymic sections, prepared several weeks after reconstitution began, to tell which T cells had originated from which stem cells. The results were similar to those that had been previously obtained using a chromosome marker⁴: often thymus lobes contained no cells of congenic type and in other cases discrete foci of congenic-type cells were seen, and occasionally an entire thymus lobe was uniformly of that type. This pattern of distribution indicates that the thymus is recolonized by very few 'stem' cells, and sometimes by only one. As Ezine *et al.* point out, although this model does not tell us the actual number of stem cells colonizing a thymus throughout embryonic development, it does suggest it could be small compared with the size of the T-cell specificity repertoire. That argues in favour of the generation of receptor diversity within the thymus.

The second issue raised by the study is the long-disputed relationship between the thymic cortex and medulla. The medullary region of the thymus contains most of the organ's phenotypically and functionally mature cells (about 10 per cent of the total cell population). The simplest interpretation is that these are the immediate source of cells destined for export, although there could be a similar, but small and transient, pool of such cells in the cortex. The cortex contains the majority of dividing cells and the majority of thymocytes, most of which are both non-functional and phenotypically different from mature T cells. The simplest interpretation would be that these are the immature precursors of the medullary population, but this does not accord with the intrathymic death of most cortical cells. The medullary and cortical populations in fact display a large degree of independence in cell kinetic studies^{5,6}.

Nevertheless, Ezine *et al.* report cases where both cortex and medulla appear to be repopulated by a single clone, suggesting that a single stem cell has initiated a cell lineage which populates first the cortex and then the medulla. A subpopulation of apparently cortical blast cells, capable of reconstituting both cortical and medullary compartments, was reported by B.-J. Fawkes and B. Mathieson at the meeting on T-cell differentiation at the Basel Institute for Immunology last November. This could be a later intrathymic stage of the type of bone-marrow stem cell suggested by Ezine *et al.* Note, however, that Ezine *et al.* also report instances where only the medulla showed reconstitution with congenic-type cells, suggesting a direct seeding of the medulla without cortical

involvement. The studies of Joterau and Le Douarin⁷ on colonization of the avian embryonic thymus also suggest independent seeding of the cortex and the medulla.

Why there might be two separate developmental pathways, one cortical to medullary, one medullary alone, is far from clear. It would make more sense if T-cell sublineages of different function were handled in different parts of the thymus. With our present knowledge, it would be more sensible and comprehensible if all T-cell development, from stem-cell immigrants to functional T-cell emigrants, took place in the medulla. It is the involvement of the cortex, with its high level of both cell generation and cell death, and with its non-functional lymphoid cells

of peculiar surface composition, that forces us to acknowledge there is something interesting happening in the thymus that we still do not understand. □

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Earth science

Anomalous satellite motion and mantle viscosity

from Kurt Lambeck

FOR many years geodesists have examined evidence for the temporal changes in the Earth's global shape and gravity field. Confirmation that such changes occur on time scales of at least several years has now emerged from the observation of a secular acceleration $\dot{\Omega}$ of the node Ω of the orbit of the satellite Lageos^{1,2}, launched by NASA in 1976 and tracked ever since with high-precision laser-ranging equipment. Acceleration of the node implies a secular change in the flattening of the Earth and the most obvious explanation of this — the mass readjustment inside the Earth associated with the rebound of the crust after the melting of the Late Pleistocene ice sheets^{1,2} — has recently been analysed in detail by Wu and Peltier³.

One benefit of the measurement of the anomalous orbital motion is that it will provide an additional estimate of the viscosity of the mantle, a quantity that is central to discussions of mantle convection yet remains uncertain. The information on viscosity contained in the observation of $\dot{\Omega}$ is the same as that contained in the observation of the Earth's secular acceleration but the latter is contaminated by other physical processes, most notably the secular tidal contributions; as a result no accurate figure for the non-tidal acceleration is available. Furthermore, non-tidal acceleration cannot be uniquely associated with the post-glacial rebound; for example, very long-period contributions could arise from electromagnetic coupling forces acting at the core-mantle interface. The orbital perturbation in the node may therefore reflect a more direct measure of the mantle's effective viscosity.

The power of the analysis of satellite orbits to determine the dynamic flattening, J_2 , of the Earth with high precision is well known⁴. Nonetheless, observations of

temporal fluctuations in this quantity have proved elusive. What was missing, until Lageos, was a satellite that could be tracked with great precision and whose orbital motion was almost entirely governed by the Earth's gravity field.

The two studies use nearly the same Lageos data and there are many similarities in the orbital analyses used. Yet their results for the secular accelerations differ significantly: Rubincam² obtains $(-10.8 \pm 2.4)10^{-3}$ arc s yr⁻² as the value of $\dot{\Omega}$ while Yoder *et al.*¹ obtain $(-14.7 \pm 1.3)10^{-3}$ arc s yr⁻². Are we in for another long debate on the correct value of a secular variation, matching that of the Earth's non-tidal secular rotation? Why do these differences occur? The problem may be partly one of contamination by long-period tidal contributions because superimposed upon the secular motion of Ω are long-term periodic fluctuations due to the tidal deformations of the Earth, principally the 18.6 year tide. The periods of these tides are well known but the amplitudes are not, primarily because both the ocean response and departures of the solid Earth's response from that of an elastic body remain uncertain⁵. Thus with only 5.5 years of data the Lageos results are still very preliminary. In more leisurely times the authors would have patiently waited until a much longer record had been painstakingly acquired, but one cannot be sure that NASA will still be tracking Lageos a few years from now.

Whatever the exact answer, Wu and Peltier believe it can be explained as the response of the Earth to the new surface load following the removal of the Pleistocene ice from the glaciated continents and the addition of an equal water-mass to the oceans. Locally, sea level and gravity are changing through time; globally the Earth's rotation and the node of satellite

orbits undergo secular changes. Many attempts have been made to deduce mantle viscosities from these direct and indirect observations of the post-glacial rebound but the results have remained conflicting. What can be said is that nearly all observations are accommodated by mantle models of uniform Newtonian viscosity of 10^{21} – 10^{22} P. Where controversy arises is in whether there is a significant depth-dependence of viscosity, whether this viscosity is linear and whether there is evidence for lateral variations in viscosity⁶. A principal reason for this state of affairs is that the results are only as good as our inadequate knowledge of the distribution and melting history of the ice loads themselves, partly determined by the rebound observations, particularly by observations of sea-level changes^{7–9}.

The new J_2 or $\dot{\Omega}$ observations are largely consistent with the uniform and Newtonian viscosity range of 10^{21} – 10^{22} P. Wu and Peltier³ have attempted to combine this observation with those of the rotational response to deglaciation and conclude that the data imply there to be a thick elastic lithosphere overlying an upper mantle of viscosity 10^{22} P and a lower mantle viscosity of 1 – 3×10^{22} P. The average thickness of this elastic lithosphere is estimated to be about 120 km with the continental layer being about 200 km thick. This conclusion is reached by assuming a viscosity profile that is determined by local observations of rebound¹⁰ and then determining the lithospheric thickness from the rotation data. The conclusion therefore remains dependent on the assumed ice history. Other recent studies^{7–9} have shown that the proposed melting histories remain inadequate to explain sea-level changes in tectonically stable regions far away from the ice loads and point to mantle viscosities that are less than 10^{22} P. As long as the conclusion of a thick average lithosphere is not dependent on the assumed ice load, these observations raise the interesting possibility that there are substantial regional variations in viscosity, for the far-field Holocene sea-level fluctuations are responsive mainly to the mantle flow induced by the added melt-water rather than the flow under the former icesheets. □

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Oncogene activation

Message of *myc* in context

from Miranda Robertson

THERE are two reasons for the exceptional concentration of research on the cellular *myc* proto-oncogene (*c-myc*). The first is historical. The chromosomal translocations characteristic of Burkitt's lymphomas transfer *c-myc* from its normal context into an active immunoglobulin locus¹, where it was quickly identified by a number of research teams whose vigorous investigations on the molecular basis of antibody diversity were immediately deflected to the molecular basis of cancer. The second is the nature of the *c-myc* gene itself. Unlike many other oncogenes, *c-myc* is expressed in a wide variety of both normal² and tumour³ tissues, and can therefore be expected to have some general significance. This expectation is borne out by two recent papers directly implicating *c-myc* in the control of normal and abnormal cell growth^{4,5}.

The implication of all the recent research, broadly, is that what changes during tumorigenesis is not *c-myc* itself but the regulation of its production. The evidence comes partly from the two recent papers^{4,5} on changes in *c-myc* expression during the growth cycle of tumorigenic and non-tumorigenic cells *in vitro*, and partly from the latest investigations on the interaction of *c-myc* with the regulatory mechanisms operating on the immunoglobulin locus in Burkitt's lymphoma cells.

c-myc and the cell cycle

The data on the cell-cycle regulation of *c-myc* come from four laboratories: those of Philip Leder and Charles Stiles⁴, and those of Gail Sonnenschein and Arthur Pardee⁵. The aim of their experiments was to establish where the product of the *c-myc* gene might act in the successive steps by which cells progress through their growth cycle. There is evidence, cited in both papers, that the progression from quiescence to DNA synthesis (that is, through G_0 – G_1) can be divided into roughly three phases, each triggered by different tissue-specific growth factors. The first phase is known as the phase of priming for growth competence; and it is in this phase that *c-myc* is implicated.

Kelly *et al.*⁴ have been able to show that a fibroblast mitogen, platelet-derived growth factor (PDGF), and two lymphocyte mitogens, concanavalin A and lipopolysaccharide, stimulate the expression of *c-myc* in those cells that normally respond to them. All three mitogens are believed to act early in the cell cycle to induce competence; and all stimulate transient expression of *c-myc* within 1–2 hours, with a return to baseline well before the onset of DNA synthesis.

The activation of *c-myc* by PDGF is particularly noteworthy, and indeed is particularly noted by Kelly *et al.*, in the light of the very close relationship between the sequence of one chain of PDGF and that of the product of the *c-sis* oncogene^{6,7}. This may be the first clear illustration of how the cellular proto-oncogenes encode different components of a single regulatory chain, disruption of any part of which could contribute to tumorigenesis.

The implication of the Campisi *et al.*⁵ data is that after a normal cell has been transformed into a tumour cell, regulation of the *c-myc* gene may be disrupted in such a way that its activation is independent of growth factors. They have measured *c-myc* expression during growth and quiescence in three cell lines: the non-tumorigenic mouse fibroblast line A31, and two tumorigenic derivatives, BPA31 and DA31, both transformed by chemical carcinogens. The growth of all three lines can be arrested by starvation (though the tumorigenic cells are slower to respond); but whereas arrested A31 cells cease to express *c-myc*, the transformed cells continue to express the oncogene at the same level after arrest. Apparently, *c-myc* expression, and thus growth competence, is no longer subject to regulation by serum growth factors after transformation.

These observations are, as Campisi *et al.* point out, consistent with the existing evidence that the activation of *c-myc* depends on the disruption of the genetic control of its expression, and not with mutations affecting the function of the product. (This is by contrast, for example, with the *c-ras* family, at least some members of which can be activated by mutations in their coding sequence^{8–10}.) They are not, as it happens, particularly consistent with the notion, derived from the transfection experiments of Land *et al.*¹¹, that *c-myc* is one of a class of oncogenes whose activation immortalizes cells (A31 cells are already immortal) and that a second gene (for example one of the *ras* family) is needed to complete transformation. But then, this categorization is in any case operational and indeed Land *et al.* explicitly state that they have no direct evidence that *c-myc* transfection immortalizes. What all this probably means is that tumorigenesis is a function of interactions between different oncogenes (in the Land *et al.* experiments *c-myc* and *c-Ki-ras*) that need not be the same in all tumours. There is, for example, no way of knowing whether the deregulation of *c-myc* in BPA31 and DA31 cells is due to a change in the regulatory region of the gene itself, or to the deregulation of some other component of the pathway in which *c-myc* par-

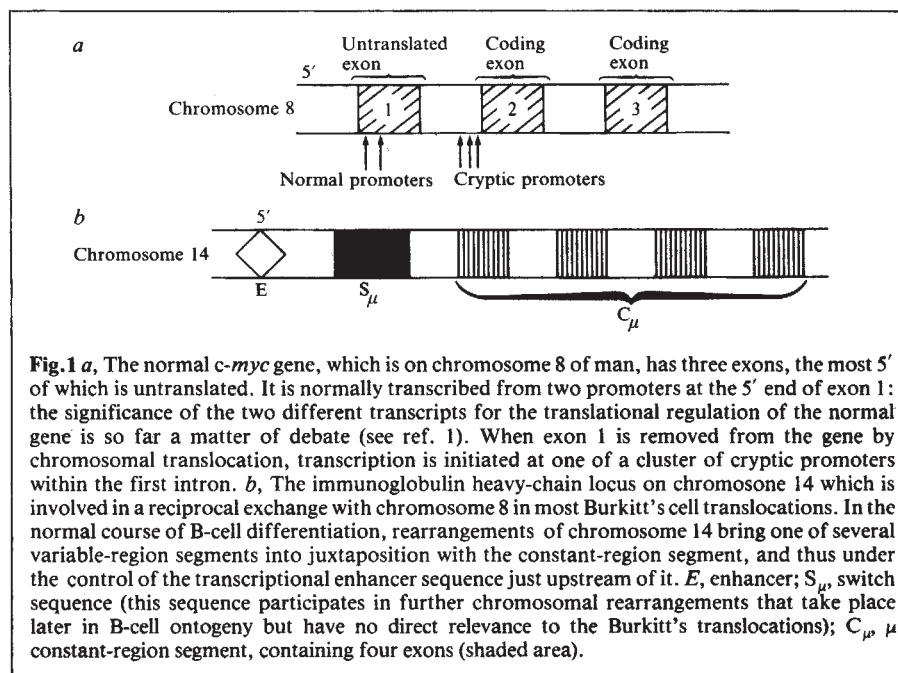


Fig. 1 *a*, The normal *c-myc* gene, which is on chromosome 8 of man, has three exons, the most 5' of which is untranslated. It is normally transcribed from two promoters at the 5' end of exon 1: the significance of the two different transcripts for the translational regulation of the normal gene is so far a matter of debate (see ref. 1). When exon 1 is removed from the gene by chromosomal translocation, transcription is initiated at one of a cluster of cryptic promoters within the first intron. *b*, The immunoglobulin heavy-chain locus on chromosome 14 which is involved in a reciprocal exchange with chromosome 8 in most Burkitt's cell translocations. In the normal course of B-cell differentiation, rearrangements of chromosome 14 bring one of several variable-region segments into juxtaposition with the constant-region segment, and thus under the control of the transcriptional enhancer sequence just upstream of it. *E*, enhancer; *S_μ*, switch sequence (this sequence participates in further chromosomal rearrangements that take place later in B-cell ontogeny but have no direct relevance to the Burkitt's translocations); *C_μ*, constant-region segment, containing four exons (shaded area).

ticipates: it might even reflect changes in *c-sis*.

It is in establishing a precise mechanism for *c-myc* deregulation that Burkitt's lymphoma cells may be important. In those cells, the *c-myc* gene on the normal chromosome 8 is silent, while its allele, which has been translocated to an immunoglobulin gene locus, is constitutively active. Thus deregulation must be associated with the translocated gene itself, or its flanking DNA. The latter could be either from the original context of *c-myc* on chromosome 8 or from its new context in the immunoglobulin locus (usually on chromosome 14).

Mutation and derepression

There have been, very broadly, two schools of thought on the mechanism of *c-myc* activation in Burkitt's cells. The first, which derives from George Klein's original observations on Burkitt's translocations¹², is that the answer lies in the constitutive activity of the immunoglobulin locus in the lymphoma cells. While everyone agrees that the immunoglobulin locus must play some part in *c-myc* activation, it has proved remarkably difficult to implicate any specific immunoglobulin regulatory mechanism: various abortive attempts have been summarized in an earlier article¹. The alternative is that the *c-myc* gene and its own regulatory sequences are altered in the translocation. There is fresh evidence for both schools of thought.

One possibility that can almost certainly be ruled out is that *c-myc* is activated by mutations in its coding exons. The *c-myc* gene has three exons (Fig. 1*a*), of which only exons 2 and 3 are translated^{13,14}. Exon 1, which is highly conserved, is suspected of subserving a regulatory function. In no case is either exon 2 or exon 3 broken in the Burkitt's cell translocations; and with one exception (which will be discussed later) no

mutations have been found in the translocated coding exons (ref. 15 and C. Croce, personal communication). Attention has thus recently been focused on the putative regulatory exon, exon 1. The issue is the importance of the loss of *myc* regulatory regions relative to that of acquisition of immunoglobulin regulatory elements, in the activation of the translocated *c-myc* gene.

The evidence in favour of a regulatory function for exon 1 of *c-myc* is strong but circumstantial. Exon 1 is highly conserved across species in the cellular gene, but entirely absent from the *v-myc* oncogene of tumour viruses. This strongly implies that it is vital to the normal function of the gene but unnecessary or even inimical to its transforming function. Leder and his colleagues have put forward the theory that the 5'-untranslated region including exon 1 is the binding site for a transcriptional repressor, possibly induced by, or even identical to, the *c-myc* product itself¹⁶. This kind of regulatory feedback could clearly account for the transience of *c-myc* activation by growth factors; and Leder and his collaborators have bolstered their case by showing that when protein synthesis is inhibited by cycloheximide, transcription of *c-myc* in response to growth factors is prolonged⁴.

The circumstantial case is further strengthened by the chromosomal translocations that are typical of mouse plasmacytomas. Like those in Burkitt's lymphomas, they almost always move *c-myc* to the immunoglobulin heavy-chain locus (see Fig. 1*b*); and in every case, the first exon of *c-myc* is either lost or truncated in the translocation. This suggests that loss or disablement of exon 1, or other sequences 5' to the coding exons, is associated with the production of tumours. (That the truncated gene can be transcribed at all is due to a copious supply of cryptic promoters in the first intron: see Fig. 1*a*.)

Burkitt's lymphomas, however, have failed to comply with this picture of *c-myc* activation: in many of them, the *c-myc* gene is translocated intact (see Fig. 2*a*). This has been interpreted as evidence that the constitutive activity of the immunoglobulin locus is more important than the state of exon 1 of *c-myc* in deregulating the translocated gene. Recent results from Leder's and Rabbitts's laboratories suggest an alternative, arising from a second specialized property of the immunoglobulin locus. Through a mechanism that is still not understood, antibody diversity is increased by the generation of somatic mutations in a discrete region of DNA normally occupied by the variable-region segment of the immunoglobulin gene. In Burkitt's lymphomas, this site is usurped by *c-myc*; and Rabbitts *et al.*¹⁷ have shown that in one case — that of the Raji cell line — the translocated *c-myc* gene has undergone extensive mutation. Leder and his colleagues have now used sequencing and S₁ mapping to demonstrate mutations in the intact exon 1 of two more Burkitt's cell lines¹⁸; and Rabbitts *et al.*¹⁹ have found mutations in yet another two.

If exon 1 of translocated *c-myc* genes were invariably either mutated or lost, this might be taken to resolve the activation issue in favour of the gene rather than the immunoglobulin locus. There are two complications, however. The first arises from Rabbitts's original data on mutations in Raji cells. These mutations extend into exon 2, the coding portion of the gene, where they result in 16 amino acid substitutions. It is hard to imagine that these would leave the *myc* product functionally unaffected; yet conserved *myc* function seems to be essential to tumorigenesis¹⁵.

This particular paradox is resolved by the activation, in Raji cells, of the untranslocated *c-myc* gene — which is silent in other Burkitt's lines^{19,20}. Rabbitts attributes the activity of the untranslocated *c-myc* gene directly to the mutant product of the translocated one, under Leder's repressor theory. If the *myc* product usually mediates or induces repression of the *c-myc* gene, then a mutant product may lead to a failure of repression. This leads to another paradox, however, since the normal *c-myc* gene once activated ought presumably to induce its own repression. There are various possible ways out of this difficulty, but in the present state of knowledge they are all more or less arbitrary.

The second complication arises from some data, not yet published, from Croce and his collaborators (personal communication) who have sequenced an intact translocated *c-myc* gene from one of the so-called variant translocations (see legend to Fig. 2*d*) and have found no mutations either in the coding or in the untranslated exons. This implies that the state of exon 1 may after all be irrelevant to the deregulation of the translocated gene, although alterations to other conserved sequences 5' to exon 1 cannot be ruled out.

Importance of context

Can any sense be made of the data so far? Yes, but only by disregarding for the time being the particulars of exon 1 and refocusing on the behaviour of the immunoglobulin locus, and the normal *c-myc* gene, in the course of normal B-cell differentiation. Both undergo sequential changes in transcriptional activity as the cell progresses from the proliferating lymphoblastoid stage to the terminally differentiated plasma cell in which transcriptional activation of the immunoglobulin locus reaches its peak. In lymphoblastoid cells, both the immunoglobulin locus and the *c-myc* gene are transcriptionally active; Burkitt's lymphomas represent a later stage of differentiation, in which the *c-myc* genes are probably normally silent and the activity of the immunoglobulin genes is increased. Mouse plasmacytoma cells, which have chromosomal translocations equivalent to those of Burkitt's cells, represent the terminally differentiated state, with immunoglobulin transcription at its height.

By making somatic cell hybrids between different combinations of these cell types, Croce and his associates have, in effect, been able to assay the regulatory signals acting on both translocated and untranslocated *c-myc* genes against each differentiated cell background. I have already discussed¹ the results of a series of experiments on the behaviour of untranslocated *c-myc* genes. The *c-myc* gene on the untranslocated chromosome 8 in Burkitt's cells is silent in the lymphoma cell but transcriptionally active in a lymphoblastoid cell background. The normal active *c-myc* genes of the lymphoblastoid cell, by contrast, are silenced when transferred to a mouse plasmacytoma cell. This presumably reflects the influence of stage-specific regulatory factors acting on the normal regulatory sequences of the *c-myc* gene.

In more recent experiments along the same lines²¹, Croce and his collaborators have gone on to analyse the stage-specificity of transcriptional activation of the translocated *c-myc*, whose activity presumably reflects the normal regulatory influences on the immunoglobulin locus. Indeed, by choosing a Burkitt's cell line in which the translocation has broken both the *c-myc* gene and the immunoglobulin locus, they have been able to use the reciprocal translocations (Fig. 2*b* and *c*) to assay separately the regulatory elements of the two separated parts of the immunoglobulin locus on the separated parts of *c-myc* in two different backgrounds: lymphoblastoid and plasmacytoma.

The Burkitt's cell line that Croce and his co-workers used is ST486, in which there is a reciprocal translocation between chromosome 8, which contains the *c-myc* locus, and the immunoglobulin heavy-chain locus on chromosome 14. The break on chromosome 8 falls between exon 1 and exon 2 of *c-myc*, the two coding exons being transferred to the immunoglobulin locus on chromosome 14 (see Fig. 2*b*) while

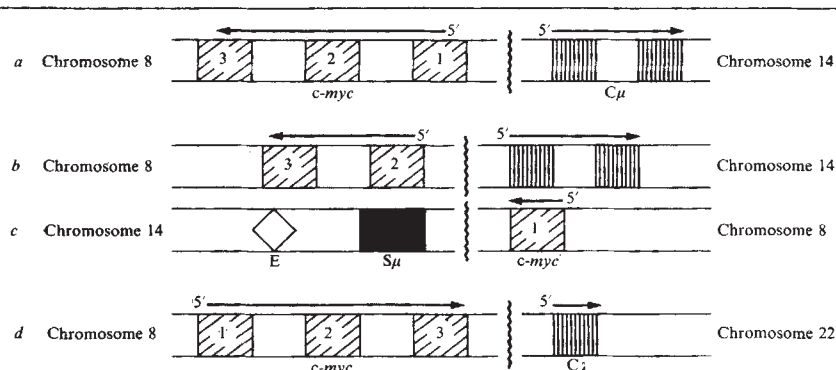


Fig. 2 *a*, Typical translocation with *c-myc* intact; *b*, typical translocation with *c-myc* separated from its exon 1; *c*, reciprocal of translocation shown in *b*. The typical translocation in Burkitt's lymphoma cells involves a reciprocal exchange between chromosome 8 and chromosome 14, resulting in the juxtaposition of the *c-myc* gene with the immunoglobulin heavy-chain constant-region locus *Cμ*, the two genes being in reverse transcriptional orientations (shown as 5' →). The distance of the chromosome 14 breakpoint from the constant-region locus is extremely variable; the breakpoint on chromosome 8 also varies, sometimes separating exon 1 of *c-myc* from the coding exons (*b* and *c*) and sometimes not (*a*). *d*, In the rare variant translocations, the translocation is between chromosome 8 and the immunoglobulin light-chain loci on chromosome 22, as shown here, or on chromosome 2. In these cases *c-myc* remains intact on chromosome 8 and is joined to the constant-region locus of one of the two light chains, tail to head, so that both are in the same transcriptional orientation.

exon 1, left behind on chromosome 8 (Fig. 2*c*), is joined to a fragment of the immunoglobulin locus containing the one known tissue-specific regulatory sequence operating on the immunoglobulin genes — the enhancer sequence upstream of the constant-region segment (see ref. 1).

One of the many earlier reverses in the attempt to understand *c-myc* activation in Burkitt's cells was the discovery that this transcriptional enhancer is usually separated from the immunoglobulin locus in the translocation, and thus cannot account for the transcriptional activation of the invading *c-myc* exons. Since the *c-myc* exons are activated notwithstanding, it became clear that there must be some other transcriptional activator operating on the immunoglobulin locus. Moreover, because some of the translocations place *c-myc* far away from the constant-region segment, it must operate over a very long range. Croce now believes he has indirect evidence of just such a long-range enhancer.

In hybrids between ST486 and lymphoblastoid cells, in which as a general rule the immunoglobulin genes of both parent cells are expressed, the translocated *c-myc* gene of the ST486 cell is silent — despite having lost its first exon, and thus being freed from any *myc* repressor. Apparently, this gene is not responding to the normal tissue-specific activation signals either for *c-myc* (normally active in lymphoblastoid cells) or for the immunoglobulin genes. Croce interprets this to mean that the putative factors responsible for transcriptional activation of the immunoglobulin locus in lymphomas are absent from the lymphoblastoid cell background; in other words, the activation of the putative long-range enhancer is strictly stage-specific.

The evidence for stage-specificity is strengthened by the behaviour of the reciprocally translocated exon 1 in plasmacytoma cells. In its original Burkitt's lymphoma

background, the isolated exon 1 is transcribed, presumably under the influence of the identified immunoglobulin enhancer to which it is tail-to-tail. In plasmacytomas, however, it is silent, while translocated exons 2 and 3 of *c-myc* are transcribed. This is presumably because the enhancer is 'read' only at earlier stages of B-cell differentiation, the longer-range mechanism taking over at the plasma-cell stage and accounting for transcription of the translocated coding exons.

If I had to guess, I should say that exon 1 of the *c-myc* gene will turn out to be critical to the cell-cycle regulation of *c-myc* in its normal context; and that in Burkitt's cells, tissue-specific immunoglobulin gene activators simply override all local *c-myc* controls that might otherwise influence the translocated gene¹⁶. In short, from investigating *c-myc* regulation in lymphoma cells, we may learn more about the regulation of immunoglobulin genes than about the control of *c-myc* expression. □

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Archaeopteryx palaeontological myopia

SIR — Howgate¹ criticizes our interpretations^{2,3} of the homologies and morphology of the *Archaeopteryx* manus by distorting and misrepresenting our viewpoints. Palaeontologists have identified the digits of theropods, *Archaeopteryx* and birds as digits 1-2-3 of the primitive reptilian hand whereas embryologists⁴⁻⁷ have identified the bird digits as 2-3-4. Do the similarities observed by palaeontologists have greater weight than the evidence derived from ontogeny by embryologists?

The correspondence of the number of phalangeal elements in the manus of *Archaeopteryx* with the primitive reptilian formula has convinced palaeontologists that the digits of *Archaeopteryx* represent the digits 1-2-3. We stated that the digits of birds must be 2-3-4 based on embryological work^{4,5}. If *Archaeopteryx* is a bird, it should have digits 2-3-4 and not 1-2-3 as suggested by Howgate¹.

Howgate¹ states that we^{2,3} resurrected "a broken phalanx hypothesis" proposed by Heinroth⁸ which states that there was a break in the proximal phalanx of topographic digit 3 and that we "arrived at a non-theropod phalangeal formula (2-3-3)". We cited Heinroth⁸ because it was essential for a complete review. We² stated that "The break or joint in the first phalanx of the fourth digit appears immediately distal to the immobilized area". We again stated³ (p.144) that "either a break occurred in the first phalanx (of the last digit) or if there are two phalanges a distortion of their orientation". We did not conclude that the phalangeal formula of *Archaeopteryx* was 2-3-3, but only stated the possibility of there being a break. Contrary to Howgate¹, only the Berlin and Maxberg specimens have the essential areas of the manus preserved as bone. Their state of preparation precludes a definitive statement of the phalangeal formula of *Archaeopteryx*.

In the left manus of the Berlin specimen there is a laterally directed flange on the first phalanx of the middle digit, directed to the junction of the supposed joint or break. Howgate implied that we² said that this flange "served to brace and strengthen the wing"¹. Howgate¹ further states that "the 'flange' appears to be a secondary outgrowth due to injury or disease and not an original morphological structure". We did not state that this flange could serve to brace the wing since such a small structure cannot brace an entire wing. This functional misconception can only be attributed to Howgate. His illustration of the right manus demonstrates that this area is covered by matrix and a digit. Therefore how can he conclude the flange is an anomaly or a normal structure?

Attempting to make our explanation appear ludicrous, Howgate¹ implies that our "scenario" of the preservation of the fourth digit (its crossing under the third

digit) was the result of *Archaeopteryx* "crossing its fingers hopefully as it plunges at high speed in the sea wing-tips first". We stated (as a scenario) that the break or joint in topographic digit 3 was caused by forces during stalling^{9,10}, impact upon the water, or as a result of preservation. Howgate¹ also stated that "all the specimens of *Archaeopteryx* with a well preserved manus have the second and third digits crossed". This is not the case since the Maxberg specimen¹¹ has a manus without this condition.

The important aspect of the homologies of the avian manus is the debate between the 1-2-3 and 2-3-4 hypotheses and the implication that different developmental patterns in digit reduction can produce similar morphologies.

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Evolutionary unease?

SIR — Peter Fellgett raises an interesting suggestion¹ that the study of cybernetic feedback loops throws light on the redundant (multiple copy) nature of functional genes and hence places the evolution of this phenomenon within the rubric of Darwinian natural selection. His proposition is that selection has favoured organisms in which gene redundancy not only protects them from mutation but also leads to enhanced prospects for survival in that 'a new [biological] program would run rather than crash'. He considers this system of 'selection for selectability' as more acceptable than so-called alternative *ad hoc* suggestions such as molecular drive, which he thinks should only be invoked as additional evolutionary mechanisms if the necessities were demonstrated; the implication being that selection is our basic null hypothesis.

I have no objection to Fellgett's basic proposition, although I cannot understand his premise that the scientific necessity for alternative mechanisms depends on which side of the Darwinian fence they fall. Molecular drive² is based on several well-known processes of non-reciprocal exchange between redundant genes, all of which have the interesting and biologically important effect of continually replacing a family of

genes with newly-arising mutant genes, throughout a sexual population. This mechanism of spreading a mutant gene, or parts of genes, can explain the widespread observation of clear differences between species in the way in which families have become replaced (homogenized). The phenomenon has been observed in families of both protein-coding and RNA producing genes and in non-functional DNA families, generally irrespective of the degree of redundancy or chromosomal dispersion of the families. Hence it is extraordinarily difficult to explain by selection alone the parallel homogenization of hundreds of families and the accidental gain and loss of complete subfamilies and families, across any one species, without invoking generally implausible *ad hoc* scenarios. Genetic turnover potentially constitutes an independent mechanism of population change with respect to both the *de novo* origin and the subsequent behaviour of redundant genes. Selection and drift can be expected to interact with this mechanism, depending on the gene family and population structure in question (see refs 2-9).

I am quite comfortable with Fellgett's idea that the origin of redundancy (and I would add the existence of nonreciprocal exchange processes within the redundant families) might be due to selection via cybernetic multiple feedback loops, that is, the processes underlying molecular drive have arisen via natural selection. There is nothing in evolution that cannot be explained by natural selection, if that is our starting point. However, this possibility would need to take into account why so many hundreds of qualitatively different genic and non-genic DNA families exist, why the degree of redundancy varies over several orders of magnitude both for the same family between species and for different families in the same species, why many families differentiate into multiple subfamilies, and why there are continual rounds of family homogenization by variant genes or parts of genes across a species. An unsubstantiated appeal to a seeming Darwinian "selection for selectability" based on cybernetic theory is not a sufficient justification for discounting the necessity for an additional evolutionary mechanism which is based on known DNA turnover mechanisms within the genome. That molecular drive is non-Darwinian is surely irrelevant.

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The active site of acidic aluminosilicate catalysts

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The availability of solid acidic catalysts based on zeolite ZSM-5 make possible observations on the nature and the absolute rate behaviour of the individual protonic sites. Tetrahedral aluminium atoms are the highly reactive ingredients, even at levels of parts per million, or less. Turnover numbers for several hydrocarbon reactions equal and exceed familiar enzymatic turnover values. Intensive catalytic activities can result from aluminium levels likely to be ignored by the experimenter.

THE acid catalytic activity of aluminosilicates is usually assumed to reside in sites involving tetrahedral aluminium atoms in substitutional positions in the framework of silica; the negatively charged aluminium sites are associated with cations which, in the case of acid catalysts, are protons. It has, however, been difficult to identify and count the number of active sites, and thus to determine quantitatively the turnover rate of which these sites are capable. With the availability of zeolitic catalyst, and particularly of ZSM-5 catalysts, such a quantitative description is now possible.

For many decades, acidic clays and amorphous silica-alumina compositions have been used to catalyse the cracking of carbon-carbon bonds, for alkene isomerization and polymerization, aromatic alkylation with alkenes or alcohols, transalkylation, and other acid-catalysed reactions. The cracking of alkanic bonds requires the highest activity; alkene transformations require lower catalytic strength and/or lower temperature.

Activity of aluminosilicates

A convenient measure of activity is based on the alpha (α)-test^{1,2} which has been refined³. It consists of measuring the first-order rate constant for the cracking of *n*-hexane at 538 °C at 100 mm Hg. For comparing relative activities, $\alpha = 1$ corresponds to the activity of a high surface area (420 m² g⁻¹) amorphous silica-alumina catalyst (10 wt% Al₂O₃ content), which has a first-order rate constant for *n*-hexane cracking of $k = 0.067$ s⁻¹. This corresponds to a reaction rate ($dn/dt = kc$) of 1.3×10^{-7} mol s⁻¹ g⁻¹ of catalyst characteristic of highly active catalyst particles in commercial petroleum cracking.

By definition, k is the inverse of a residence time (for 63% conversion). Previously², we have used the superficial residence time, based on vapour flow rate into the reactor measured at room temperature, and the bulk catalyst volume; this gave a rate constant of 0.016 s⁻¹. This is convenient when relative activities (α) are to be determined for practical reactor operations. To describe an absolute rate, we use the more accurate value based on vapour flow in reaction conditions (538 °C) and on unit weight of catalyst.

As the above catalyst contains about 0.002 mol of Al g⁻¹, the turnover rate above corresponds to only 4×10^{-3} molecules min⁻¹ per atom of total aluminium in the sample. However, the fraction of the Al atoms which are both accessible and in the proper chemical condition (tetrahedral substitution in silica structure) is unknown.

With the zeolites as catalytic solids, we have progressed from solids with a, usually unknown, number of active sites available on the geometric particle surface to an essentially 'transparent' medium: all intra-crystalline sites can be available for molecular contact.

With zeolite Y in the hydrogen (protonic) form, α activities of between 1,000 and 5,000 and higher were observed^{1,2}. This implies a turnover capability of the order of 2–10 molecules min⁻¹ per Al atom. However, the equivalence and

degree of participation of all Al atoms was not clear in view of the high aluminium atom density in this zeolite, approximately one Al atom for two Si atoms, and the ease with which aluminium can undergo hydrolysis and removal from the framework.

Physical chemistry of ZSM-5

In contrast to zeolites previously used as catalysts, the aluminium atom concentration in zeolite ZSM-5 is very low and continuously variable over many orders of magnitude, as illustrated by Fig. 1. The representation of aluminium concentration when expressed in terms of the SiO₂/Al₂O₃ ratio traditionally adopted to characterize zeolite mineral structures, demonstrates the variability and unique diluteness of Al-atom concentration achievable for ZSM-5.

The factor of most physical chemical significance is that, in ZSM-5, Al atoms are no longer structure-determining constituents, that is, they no longer contribute a major portion of the framework structure³.

The average distance between aluminium sites is equivalent to several to many atomic diameters, and their distribution should approach randomness. It has been observed⁴ that a random model (subject only to the proviso of Loewenstein's rule) may be applicable to a zeolite at Si/Al ratios greater than a value of 2.0.

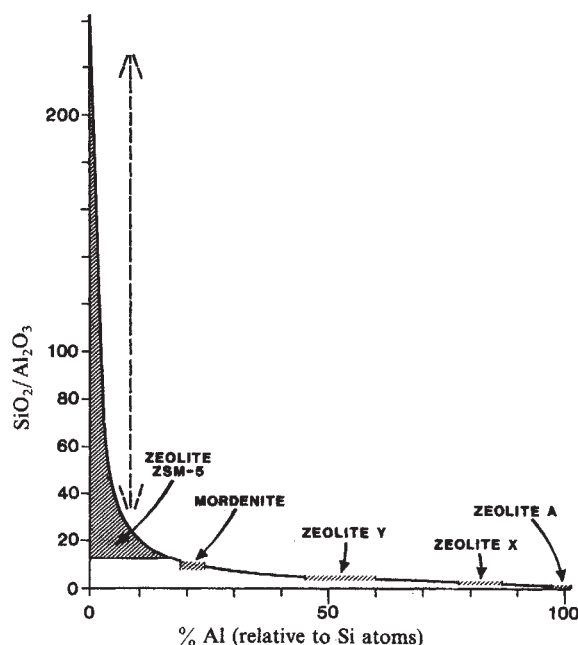


Fig. 1 Al atom concentration and SiO₂/Al₂O₃ ratio for various zeolites.

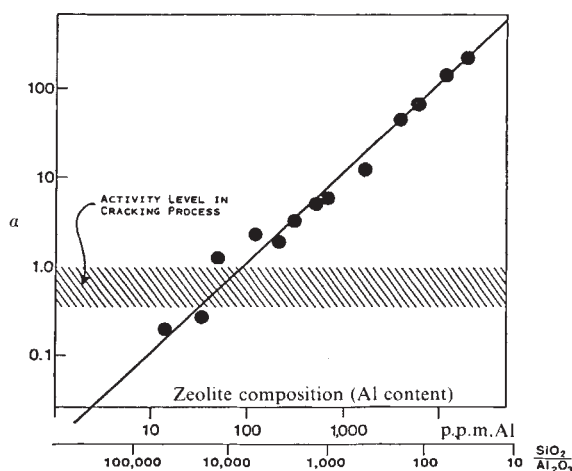


Fig. 2 The hexane cracking activity plotted against the aluminium content in HZSM-5. Shaded band indicates activities near $\alpha \approx 1$.

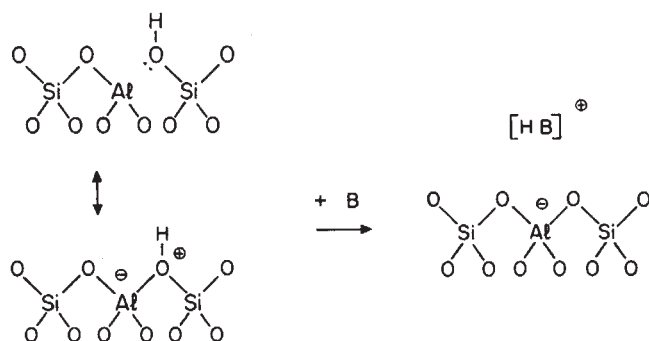


Fig. 3 The Brønsted acid site in HZSM-5 and its interaction with a base.

Elementary statistics indicate that in an assembly of 96 T-atoms (Si or Al), which define a unit cell of the ZSM-5 structure, a fraction of approximately 14% of all unit cells would have no Al atoms, when the average Si/Al ratio R of the sample is 48, according to $f = [1 - 1/(R + 1)]^{96}$. Conversely, for a ZSM-5 zeolite of a higher average Si/Al ratio of, say, 120, about 20% of its unit cells can be expected to have a Si/Al ratio of < 50 .

These calculations apply rigorously to samples with a macroscopically homogeneous aluminium distribution, both within the individual crystallites and within the sample. Some authors⁵⁻⁷ have reported Al gradients in their samples. For such samples, with average Si/Al value R , statistics will produce some deviation of Al frequency per unit cell entity in both directions.

In all cases, it will become apparent from the catalytic data that the Al atoms in ZSM-5 occur at sufficiently low concentration to be analogous to a dilute solution. There is substantial chemical uniformity of sites. (This does not rule out topologically different locations in the ZSM-5 framework, as they involve characteristic distances beyond the range of localized chemical forces.)

C-C bond cracking activity per site

The α activity is proportional to Al contents and extrapolates to zero for aluminium concentration tending to zero³. This dependence has now been extended over nearly four orders of magnitude of both activity and Al concentration, down to nearly 20 p.p.m. of Al, as seen in Fig. 2. For orientation, activities near and below $\alpha \sim 1$ correspond to levels actually used by industrial processes.

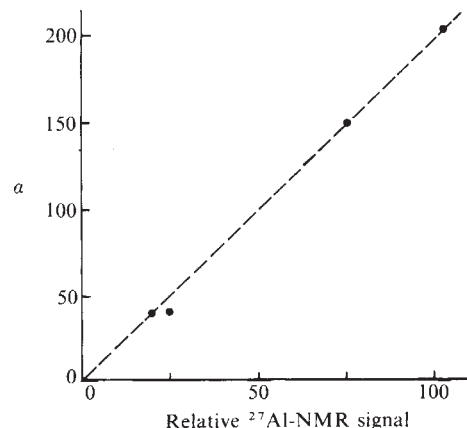


Fig. 4 The activity of HZSM-5 plotted against the tetrahedral aluminium NMR signal.

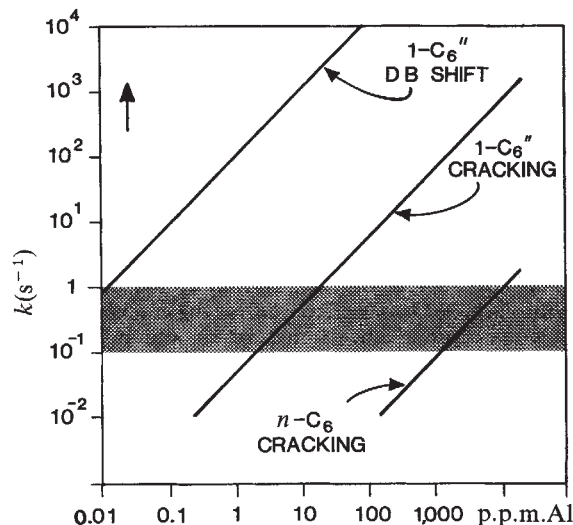


Fig. 5 Comparison of rate constants at 454 °C, of hexane cracking, hexene cracking, and hexene double-bond (DB) shift (after ref. 18).

The active Brønsted acid site in a hydrogen zeolite and its interaction with a base or substrate is depicted in Fig. 3. Magic-angle spinning (MAS)-NMR data have shown⁸ that in the dehydrated state aluminium is in a tetrahedral environment which is asymmetric, in agreement with Fig. 3 (left-hand side). On interaction with an added base, B , such as water⁸ or ammonia⁹, the aluminium is in a highly symmetrical tetrahedral coordination⁸, as indicated in Fig. 3 (right-hand side).

A meaningful quantitative correlation of activity with total aluminium content can be expected only when all Al atoms are in tetrahedral framework positions. Hydrolysis to give extra-lattice Al is frequently observed in zeolites with low Si/Al ratio such as zeolite Y^{10} . In HZSM-5, this can be readily prevented by preparing the sample in conditions that avoid undue exposure to heat and water vapour³, as has been pointed out elsewhere^{11,12}. Also, thorough replacement of sodium ion must be assured, as its concentration must be only a small fraction of the already small Al concentration.

The complete proportionality of activity and number (concentration) of Al atoms is consistent with the homogeneity of the active sites achieved in this state of dilute solution.

This and the existence and stability of a single protonic Al site in HZSM-5 is also supported by and consistent with the following independent observations:

- (1) Examination of the Al atoms using high-resolution MAS-NMR techniques¹³ has demonstrated that all aluminium incorporated during synthesis of ZSM-5 zeolite, even at the lowest level of incorporation in the synthesis, is tetrahedral in structure.
- (2) In carefully prepared samples there is a single hydroxyl band, independent of the Si/Al ratio, centred at $3,603 \text{ cm}^{-1}$ in

the IR spectrum assigned to Brønsted acid sites¹², and the intensity is proportional to Al content. A second IR hydroxyl band at about 3,720 cm⁻¹ is due to crystal terminating OH groups or to silica gel impurities^{12,14}.

(3) The ammonium form can be quantitatively reconstituted and no Lewis-bound pyridine can be found by IR¹², that is, all Al atoms in the zeolite framework are in tetrahedral coordination. (4) Measurements of the ion exchange capacities of a series of HZSM-5 samples with various Si/Al ratios show a 1:1 molar ratio of the exchanged cation, caesium, to the aluminium content³.

(5) The temperature-programmed desorption (TPD) spectrum of ammonium-exchanged ZSM-5 shows a single high-energy desorption band with $T_{\max}$ at 400 °C as observed by Kerr and Mikovsky, and others¹⁴. Kerr and Mikovsky (unpublished data) have shown that it is not composed of a multitude of bands resulting from ammonia elimination from acid sites of different acidity, as is found with HY zeolite¹⁵. The molar amount of ammonia eliminated is equal to that of the chemically determined total aluminium concentration for a series of HZSM-5 samples⁹. After exposure to excess ammonia vapour, the ammonium form desorbs ammonia from two additional lower energy states, giving rise to three desorption maxima, that are sometimes erroneously thought to indicate the presence of three types of acid site^{16,17}.

We can now demonstrate in Fig. 4 the proportionality of α activity to the tetrahedral Al concentration, as determined by MAS-NMR⁹. Clearly, the tetrahedral Al atoms (Fig. 3) and their associated protons constitute the active site.

With such quantitative data for the number of active sites available, absolute molecular turnover rates and numbers can now be determined.

The absolute turnover rate for *n*-hexane cracking at the standard test conditions (538 °C, 100 torr *n*-hexane pressure) is calculated to be about 2.8 molecules min⁻¹ per atom of Al, for the entire Al concentration range.

We find that the reaction rate is indistinguishable from first order for the entire pressure range 10–760 torr. Therefore, the fraction of occupied Al sites at the pressure of measurement (100 torr) must be <1% of all Al sites. It follows that the turnover rate per occupied active site (the turnover number) is greater than 300 molecules min⁻¹ per active Al site.

Acid-catalysed turnover rates

The intrinsic rate constants for many other acid-catalysed reactions are proportional to the α of the ZSM-5 catalysts, and therefore to the number of Al atoms; they include the rates for toluene disproportionation, xylene isomerization, alkene conversion and methanol conversion¹⁸. These rates are all greater than the alkane cracking rate.

Figure 5 compares the reaction rates we have observed for *n*-hexane cracking, 1-hexene cracking, and 1-hexene double bond isomerization. Each absolute rate was found to be proportional to tetrahedral aluminium concentration, as shown by the parallel lines. The alkene cracking rate is some 800 times the alkane cracking rate, and the alkene isomerization is some 1,300 times the alkane cracking rate.

Many commercial processes occur at a liquid hourly space velocity (LHSV) of 1–10, that is, they occur with a rate constant $k \approx 0.1$ – 1 s⁻¹. Thus, the site concentration needed for achieving such technically relevant reaction rates—a magnitude indicated

Table 1 The magnitudes of the turnover values for ZSM-5 at 454 °C, 100 torr

	Turnover rate (molecules min ⁻¹ per Al site)	Turnover no. (molecules min ⁻¹ per active Al site)
<i>n</i> -hexane cracking	0.37	>37
1-hexene cracking	290	>29,000
1-hexene double-bond isom.	370,000	$\geq 4 \times 10^7$

From data of Lago and Haag; see ref. 18.

by the shaded area in Fig. 5—can be below 1,000 p.p.m. Al for alkane conversion, and as low as a fraction of a p.p.m. to 100 p.p.m. for various other conversions such as those involving alkenes. Hence 'discoveries' of significant but unexpected catalytic activity on siliceous zeolite compositions can occur due to ignorance of the role of unanalysed amounts of lattice aluminium.

Table 1 presents the magnitudes of the turnover figures at 454 °C, expressed in the units (min) characteristic of enzymology. (These magnitudes are large and not dissimilar from those encountered in enzyme catalysis.)

Experimental procedures

The samples of HZSM-5 were prepared¹⁹ and pretreated³ according to published procedures. Total aluminium was determined by digesting the zeolite in a HF/H₂SO₄ mixture, evaporating to dryness, dissolving the residue in hydrochloric acid and, after addition of potassium chloride as an ionization and interference suppressant, analysing by atomic absorption spectrometry. The accuracy of the Al analysis depends on the concentration in the original sample with the following uncertainties: for 1,000, 100 and 25 p.p.m. Al—5%, 10% and 25%, respectively.

The concentration of framework tetrahedral aluminium was determined quantitatively by ²⁷Al MAS-NMR using a JEOL FX-200 Fourier transform spectrometer. Details of the technique will be published elsewhere⁹.

Conclusions

It has been experimentally demonstrated that the catalytic site for acid catalysis on aluminosilicate catalyst is quantitatively identifiable with a protonated tetrahedral aluminium atom in the SiO₂ framework.

The number of sites and associated acid activities have been determined, using ZSM-5 zeolites of varying Al concentration, from which absolute turnover rates and numbers are now available. These resemble in magnitude the enzymatic turnover numbers.

Careful control and quantitative knowledge of minute Al concentrations is essential in experimentation and interpretation of catalytic observations on ZSM-5 structures and other zeolites. If ignored, the results are likely to be erroneously attributed to other phenomena or components.

If we assume that the active sites in standard amorphous silica-alumina catalysts are similar in nature and specific activity to those in ZSM-5, then the above data indicate that only about 1 out of 1,000 of the analysed Al atom was effectively involved in such catalysts.

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Effect of somatic mutation within translocated *c-myc* genes in Burkitt's lymphoma

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Our previous studies of a translocated c-myc gene in the Raji Burkitt's lymphoma cell showed somatic mutations in exons 1 and 2. We have extended these observations to two other translocated c-myc genes and find a common occurrence of mutation in the noncoding exon 1. We also found that in Raji cells, unlike other Burkitt's lymphoma cell lines, the normal allele of the c-myc gene is transcribed as well as the translocated gene. These results support a model in which c-myc oncogene activation in Burkitt's lymphoma occurs by disruption of a normal transcriptional control mechanism in which the c-myc protein is itself involved.

BOTH human Burkitt's lymphoma (BL) and mouse myeloma cells display specific chromosomal translocations involving immunoglobulin gene loci and the *c-myc* proto-oncogene¹⁻⁷. In Burkitt's lymphoma there are three types of translocation involving the *c-myc* gene on the long arm of chromosome 8 (8q24). These frequently involve the immunoglobulin heavy (H)-chain locus (14q32) and less frequently, in the so-called variant Burkitt's lymphoma, involve either the κ light (L)-chain locus (2p12) or the λ L-chain locus (22q11)⁸. Somatic cell genetics⁹ and *in situ* hybridization studies¹⁰ established that in t(8; 14) the *c-myc* gene is carried into the 14q+ aberrant chromosome where it becomes associated with the H-chain constant (C)-region genes in the opposite orientation for transcription^{6,7,11,12}. Frequently this translocation to chromosome 14 does not affect the three-exon structure of the *c-myc* gene nor does the breakpoint occur near to the *c-myc* gene transcriptional promoter^{6,12}. A more dramatic variation in the breakpoint relative to the *c-myc* gene has been reported in the variant BL translocations where it occurs downstream (3') of this gene^{10,13-16}. In contrast, the analogous translocation of the mouse *c-myc* gene and the *Igh* locus very frequently results in complete or partial loss of the *c-myc* gene exon 1 (refs 2, 4, 17).

In view of this variability in the translocation breakpoint with respect to the *c-myc* gene in Burkitt's lymphoma and mouse myelomas, the possibility must be considered that, assuming the translocation activates the oncogenic potential of the *c-myc* gene, controlling elements normally involved in immunoglobulin gene transcription take control of the translocated *c-myc* gene. The possibility of higher levels of transcription resulting from translocation does not seem to be the whole answer because we were unable to demonstrate elevated levels of *c-myc* messenger RNA (mRNA) transcription in BL cell lines compared with lymphoblastoid or other tumorigenic cell lines⁶. Furthermore, a clearly demonstrable enhancer element near the human *C_μ* gene does not remain on the 14q+ chromosome in many cases of t(8; 14)¹⁸ and therefore cannot control this gene transcription in these cases. As no obvious general effect could be observed on the *c-myc* gene in Burkitt's lymphoma, we investigated the possibility that alterations in the existing elements for controlling the *c-myc* gene are important rather than the acquisition of new elements after translocation. This focused our attention on exon 1 of the *c-myc* gene, which has no assigned function, being transcribed but not translated into protein^{6,19,20} although it has been suggested to have a regulatory role²¹. Recently, we described the nucleotide sequence of the translocated *c-myc* gene from Raji cells t(8; 14) and found substantial alterations in the sequence (both base changes and deletions) of the first two exons²². The changes observed in Raji exon 2

would result in a substantially altered *c-myc* protein product, but no somatic mutations were reported to be present in another translocated *c-myc* gene sequenced recently⁷. These data suggested the possibility that changes in the sequence of exon 1, rather than coding-region segments, may be important in the activation of the *c-myc* gene. We now report the sequence of the 5' end of a Daudi *c-myc* cDNA clone⁶ which must derive from the translocated *c-myc* gene as only this allele of *c-myc* is active in Burkitt's lymphoma^{12,23}. This sequence differs from the normal exon 1 by 7% of base substitutions. Furthermore, the sequence of the translocated *c-myc* gene exon 1 from the variant BL cell line LY67 [t(8; 22)] was also found to contain base substitutions. In addition, we have observed that the normal *c-myc* gene is expressed in Raji cells (which possess a substantially altered translocated *c-myc* gene-coding segment), unlike the analogous gene in the majority of BL cell lines. This result supports a transcriptional control mechanism using, directly or indirectly, the *c-myc* gene product.

Variable translocation point

In the cell lines studied here [Daudi, t(8; 14); JI and LY91, t(2; 8); MAKU and LY67, t(8; 22)], we were unable to detect rearrangements of the *c-myc* gene using an exon 2 probe (M13J11.5Sac)⁶ in Southern filter hybridization with *Eco*RI or *Bam*HI-digested DNA (data not shown). Several *c-myc*-containing λ phage clones (see Fig. 1), were isolated from phage libraries prepared from BL DNA and a detailed restriction map of the *c-myc* locus was constructed covering approximately 8 kilobases (kb) upstream of exon 1 and 14 kb downstream of exon 3. Two subclones were prepared (pUCJ15R4 and pUCJ17BS8) (Fig. 1) and used in filter hybridizations with either *Bgl*II-digested genomic DNAs (pUCJ15R4) or *Sac*I-digested DNAs (pUCJ17BS8). The choice of these two restriction enzymes allows us to overlap the DNA segments detected by the exon 2 hybridization probe and therefore assay for possible DNA rearrangements which have occurred on the flanking sides of the probes. We only detected unrearranged restriction fragments with either probe (6.5-kb *Bgl*II fragment and 7.7-kb *Sac*I fragment) as evidenced by the similarity between all of the DNAs compared (Fig. 1). As we know that the translocation point is upstream of the *c-myc* gene in Daudi cells¹¹, these hybridization data place the translocation point at least 12 kb upstream of this *c-myc* gene, in agreement with results reported by others^{11,12}. Conversely, as the breakpoint is on the 3' side of the *c-myc* gene in variant BL cells, the lack of rearrangement present in *Sac*I-digested DNA from JI, LY91, MAKU and LY67 shows the translocation point to be >14 kb downstream of the *c-myc* gene in these cells. Due to the remarkable variability between the *c-myc* gene and the translocation breakpoint in Burkitt's lymphoma²⁴ (see Fig. 1), none of the clones obtained from the

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Fig. 1 Filter hybridization of Burkitt's lymphoma DNA using *c-myc* flanking region probes. The restriction map shown for the *c-myc* locus was generated from the λ phage derived from genomic libraries of J1 and LY67 DNA (cloned in λ 2001; ref. 34) and Daudi DNA (cloned in λ 1059; ref. 34). The extent of each of these clones is indicated below the map (λ J17 has been described previously²²). Two subclones used in this study, cloned in the pUC8 vector, were pUCJ15R4 containing a 1.2-kb *Eco*RI fragment from the 5' end of the region and pUCJ17B58 containing a 0.8-kb *Bam*HI–*Sac*I fragment. These probes were nick-translated and used in Southern filter hybridizations³⁵ of *Bgl*II-digested DNA (pUCJ15R4, a) or *Sac*I-digested DNA (pUCJ17B58, b). Hybridization conditions were $2 \times$ SSC, 10% dextran sulphate, 0.2% polyvinylpyrrolidone (PVP), bovine serum albumin (BSA) and Ficoll³⁶, 0.1% SDS, $50 \mu\text{g ml}^{-1}$ fragmented salmon DNA containing 10^6 c.p.m. ml^{-1} probe (specific activity $\sim 10^8$ c.p.m. μg^{-1}); after hybridization, filters were washed with $0.1 \times$ SSC, 0.1% SDS and autoradiographed at -70°C with prefogged film³⁷. Restriction fragment sizes were determined by comparison with λ phage DNA cut with *Hind*III. R, *Eco*RI; H, *Hind*III; Bg, *Bgl*II; X, *Xho*I; S, *Sac*I; B, *Bam*HI; BgII sites are not complete.

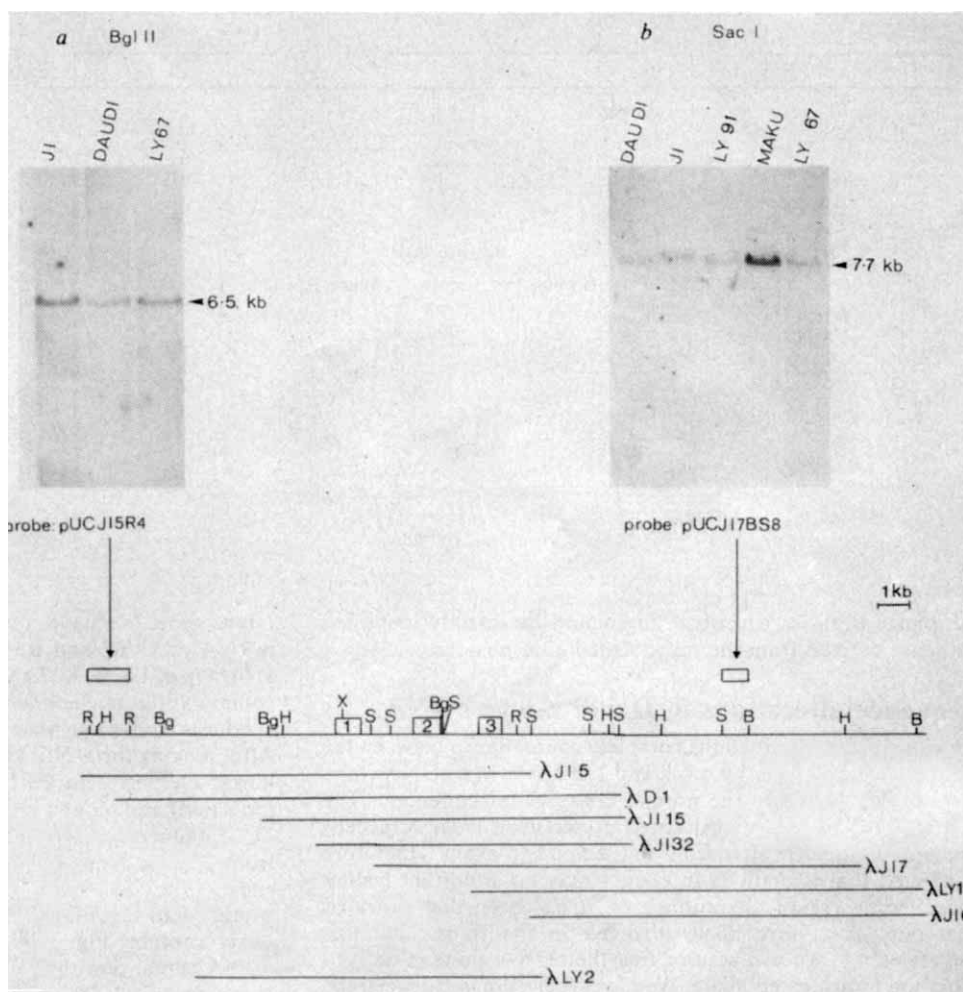
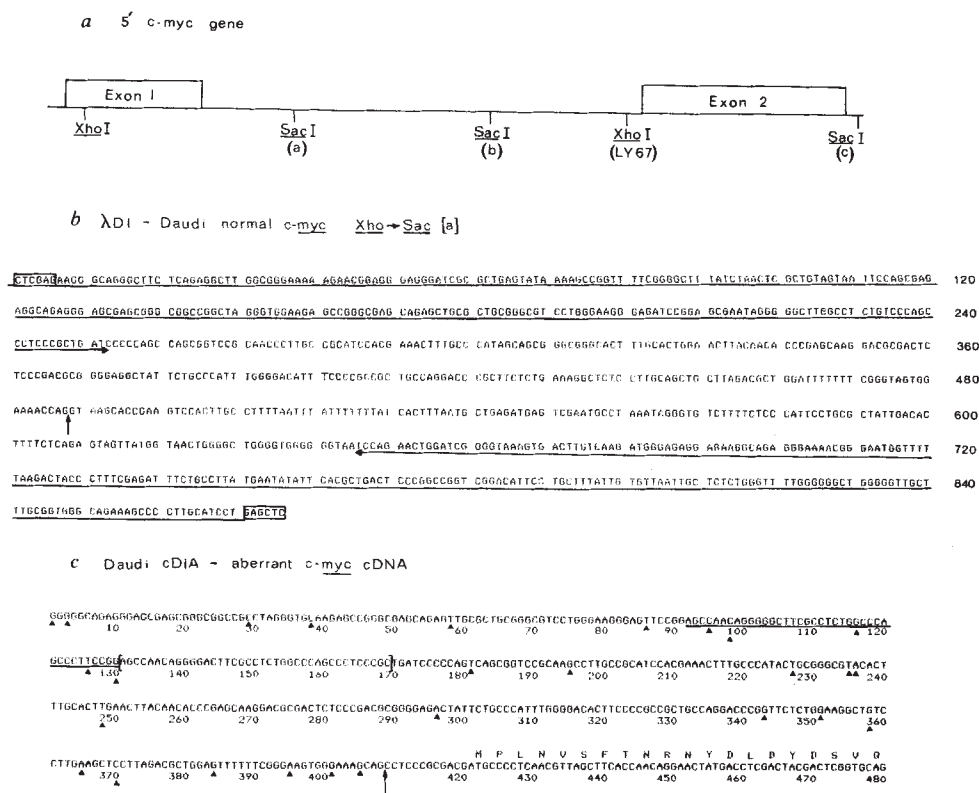


Fig. 2 Nucleotide sequence of exon 1 from the Daudi *c-myc* gene. a, Partial restriction map of *c-myc* exons 1 and 2. The *Sac*I site indicated as *Sac*I(a) is absent from clone λ LY2 and the *Xho*I site near exon 2 is unique to λ LY2. b, Exon 1 sequence of λ D1 Daudi *c-myc* gene. *Xho*I and *Sac*I sites at the ends of the sequenced fragment are boxed. The vertical arrow indicates the RNA splicing signal. The underlined regions indicate sequences obtained from the analogous region of λ J15 and λ J115. Nucleotide sequences are obtained from random sonicated fragments subcloned in M13mp8 followed by sequencing by dideoxy chain termination methods³⁸. c, 5' end sequences of Daudi cDNA clone, pUC cDIA. The sequence variations observed for pUC cDIA²² are indicated by small arrowheads (the 5' G residues may have been introduced by cloning procedures); the large vertical arrow indicates the RNA splice site. The underlined region is a duplicated segment present in the adjacent bracketed section.



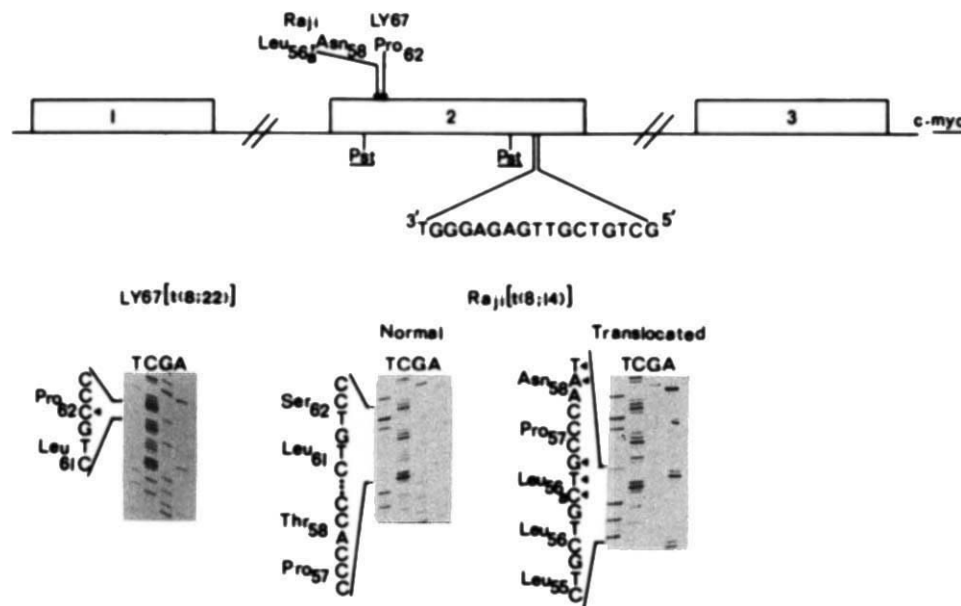


Fig. 3 cDNA cloning strategies and sequences from LY67 and Raji clones. A synthetic oligonucleotide (sequence shown beneath a diagrammatic representation of the *c-myc* genomic gene arrangement) was used to prepare double-stranded cDNA as described previously³⁹ from poly(A)-selected RNA obtained from post-nuclear supernatants. After digestion with *Pst*I, fragments were cloned into *Pst*I-digested M13mp8 and screened⁴⁰ for the presence of *c-myc* exon 2 sequences using a radioactively labelled *Sac*I fragment isolated from the clone M13J171.5*Sac*²². Positive clones were directly sequenced by dideoxy chain termination procedures. The *c-myc* gene diagram indicates the position of amino acid substitutions found in LY67 and Raji genes as well as the *Pst*I sites used in the cloning, and representative areas of sequence are shown from the cDNA clones together with the base changes (indicated by arrows) present in altered versions.

BL phage libraries described here could be directly identified as being derived from the translocated chromosome.

Sequence alterations in Daudi *c-myc* cDNA

Previously, we described the complete concordance between the nucleotide sequence of exons 2 and 3 of *c-myc* in a cDNA clone from Daudi cells and the normal *c-myc* gene sequence²². The observation that the translocated *c-myc* gene from Raji cells contained sequence alterations in the first two exons²² therefore suggested that alterations in exon 1 may be important (rather than coding region alterations). As it has been demonstrated that only the *c-myc* allele involved in the translocation is expressed^{12,23}, we can assume that the cDNA clone is derived from the translocated allele. We have, therefore, sequenced the exon 1 region of the Daudi cDNA clone and found that it contains multiple base differences compared with the sequence of exon 1 derived from the normal *c-myc* gene isolated from a phage library of Daudi DNA (included in a *Xho*I-*Sac*I fragment; see Fig. 2a). The latter sequence agrees with the exon 1 sequence of other normal *c-myc* genes^{7,22,25} and those regions underlined in Fig. 2b were also sequenced in *c-myc* clones from JI phage clones and found to contain no sequence differences. This suggests that we are not merely detecting polymorphism of the human *c-myc* exon 1 sequence. The distribution of base alterations in the Daudi cDNA exon 1 is indicated in Fig. 2c, as well as the presence of a 35-nucleotide duplicated region (starting at residue 92). The overall difference between the cDNA and normal exon 1 sequences is 7.3% (excluding the duplication) but no obvious significant pattern of these base changes is apparent from this sequence. These data argue, however, that the differences observed in the Daudi cDNA exon 1 sequence result from mutations introduced into this region during the translocation event.

Markers for translocated *c-myc*

As discussed for the data in Fig. 1, those variant BL cell lines available for study did not show obvious *c-myc* gene rearrangement when analysed by filter hybridization and therefore did not provide us with phage clones which were obviously derived from the translocated chromosome. We therefore adopted an indirect strategy for the identification of the translocated *c-myc* gene which relies on the critical observation that the normal *c-myc* allele is transcriptionally silent in Burkitt's lymphoma^{12,23}. Thus, the *c-myc* mRNA in BL cells should come from the translocated allele, and it follows that cDNA clones made from this mRNA will represent the sequence of this translocated

c-myc gene. We have, therefore, prepared cDNA clones from mRNA of LY67 and Raji cells using the procedure indicated at the top of Fig. 3. A 17-residue long oligonucleotide (designated common oligonucleotide—see Fig. 5) was used to prime cDNA synthesis from a site near the 3' end of exon 2 in the *c-myc* gene. After making the DNA double-stranded with DNA polymerase, it was cleaved with *Pst*I (for which sites occur in exon 2 as indicated) and cloned in the vector M13mp8 (ref. 26) for direct DNA sequencing. Six independent cDNA clones were made from LY67 mRNA and each of these clones showed a T to C substitution in the codon for amino acid 62. This substitution would yield a proline instead of serine at this position in the *c-myc* protein; Fig. 3 compares the relevant area of the LY67 cDNA sequence with the normal sequence derived from a cDNA clone present in Raji cells (this clone is discussed below).

The presence of a proline codon substitution in the LY67 cDNA provides a sequence marker for the translocated *c-myc* gene in this cell line. By comparing this sequence with genomic clones derived from LY67 DNA, we found that the clone λ LY2 (see Fig. 1) contained this change and therefore conclude that this genomic DNA insert was representative of the *c-myc* gene involved in the translocation in this cell. An indication of possible sequence differences at the 5' end of the genomic insert within λ LY2 was given by two restriction enzyme site polymorphisms found in the intervening sequence between exons 1 and 2. These are the acquisition of a new *Xho*I site (resulting from an A $\rightarrow$ G transition) 49 bases from the ATG codon (shown in Fig. 2a and Fig. 4) and the loss of a *Sac*I site shown as *Sac*I(a) in Fig. 2a.

The strategy for sequencing the relevant regions of λ LY2 was to subclone the 2-kb *Xho*I fragment (containing exon 1) in pUC8 and the 1.5-kb *Sac*I fragment (containing exon 2) into M13mp10, followed by fragment isolation for generation of randomly overlapping clones. A partial sequence of the segment from the 5' *Xho*I site to the *Sac*I(c) site (see Fig. 2a) of the λ LY2 insert is shown in Fig. 4. The only nucleotide alteration, compared with normal *c-myc* gene sequences, found in exon 2 is the single base change identified in the cDNA clones. Further nucleotide substitutions begin to appear in the intervening sequencing between exons 1 and 2, and multiple differences exist within exon 1 itself. Interestingly, in this case the base substitutions are confined to the 3' half of exon 1, in marked contrast to the overall spread of changes found in exon 1 of Daudi and Raji cells. Furthermore, sequence data of the promoter region of the LY67 translocated *c-myc* gene revealed no base substitutions compared with the normal sequence (unpublished results). A possible significant feature of these findings is that in three cases of translocated

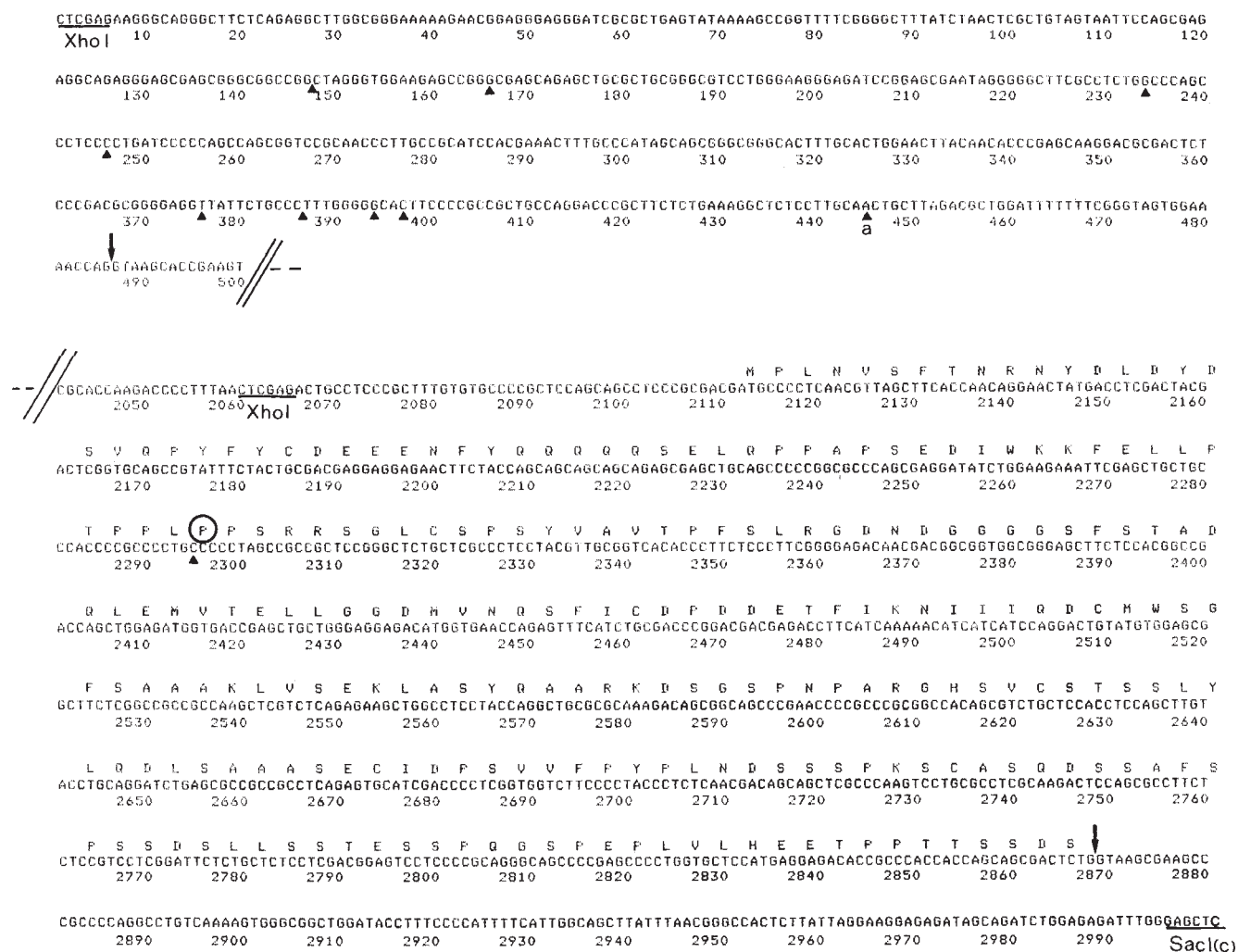


Fig. 4 Partial nucleotide sequence of the aberrant *c-myc* gene derived from λ LY2. The nucleotide sequence is shown from the *Xho*I site to the *Sac*I(c) site (Fig. 2) of λ LY2. Sequence data were obtained from random sonicated subclones in M13mp8 or subclones derived from either *Pvu*II, *Taq*I or *Alu*I digestion of a clone containing the 2-kb *Xho*I fragment (indicated in Fig. 2) in pUC8 (pUCLYXh16). Base substitutions within exon 1, compared with normal *c-myc* (Fig. 2), are indicated by small arrowheads and deletions are indicated by arrowheads between bases (deletions a and b are 2- and 3-base pair deletions respectively; the former results in the destruction of a *Pvu*II site). The proline substitution in exon 2 is circled. Restriction sites are as indicated in Fig. 2 (the *Sac*I(a) site is absent from λ LY2 and the *Xho*I site at position 2,061 is unique to λ LY2). The RNA splice sites are marked by large arrows.

c-myc genes (those of Raji, Daudi and LY67 cells), exon 1 is altered compared with the normal sequence (see below).

Normal *c-myc* is transcribed in Raji cells

Usually, the unrearranged *c-myc* gene is transcriptionally silent in Burkitt's lymphoma^{12,23}, strongly implying a direct transcriptional control of the *c-myc* gene which is, however, lost from the translocated allele. As this effect seems to be related to the product of the *c-myc* gene, it would be of considerable interest to study the effect of mutants in the *c-myc* protein on *c-myc* gene transcription. Potentially, the product of the translocated *c-myc* gene in Raji cells represents such a situation as it has been shown that exon 2 (the first coding exon) contains several codon substitutions. Therefore, we have prepared cDNA clones from Raji mRNA to investigate the possibility that the normal *c-myc* allele is active in the presence of an altered gene product. Two cDNA clones were prepared (see Fig. 3) and the sequences revealed that one of these clones derived from the translocated *c-myc* gene, as it contained the characteristic base changes present in this gene, while the other cDNA clone derived from the normal allele. Figure 3 includes relevant sequences from these clones, showing the regions of interest. The analysis of only two cDNA clones from Raji does not allow any real estimate of the relative proportions of transcription (or of translation)

from the respective *c-myc* genes. We therefore undertook to estimate the relative level of *c-myc* gene transcription in Raji cells using oligonucleotides which either detect both the normal and translocated *c-myc* mRNA (common oligonucleotide) or detect only the translocated *c-myc* mRNA (Raji-specific oligonucleotide) in Northern mRNA filter hybridizations. Figure 5 shows the results of this type of experiment; Fig. 5a illustrates the 17-long oligonucleotides used and their corresponding locations in exon 2 of *c-myc*. The common oligonucleotide is the one used for the cDNA cloning experiment and the Raji-specific oligonucleotide spans the region of sequence (illustrated in Fig. 3) which includes the additional leucine codon and the Thr→Asn substitution (position 58). (Thus, the Raji-specific oligonucleotide contains 5 out of 17 bases different from the normal *c-myc* sequence, including a 3-base insertion.) With these oligonucleotides as labelled probes for Raji or JI [t(2;8)] mRNA, we detected hybridization of *c-myc* mRNA in both mRNA populations using the common oligonucleotide (Fig. 5b) but only *c-myc* mRNA in Raji using the Raji-specific primer, thereby confirming that the latter probe only detects the Raji translocated *c-myc* mRNA in these hybridization conditions. For a quantitative estimation of the contribution of normal or translocated *c-myc* mRNA in Raji cells, the filters were rehybridized with a *c-myc* cDNA clone to allow analysis of the two sets of data by

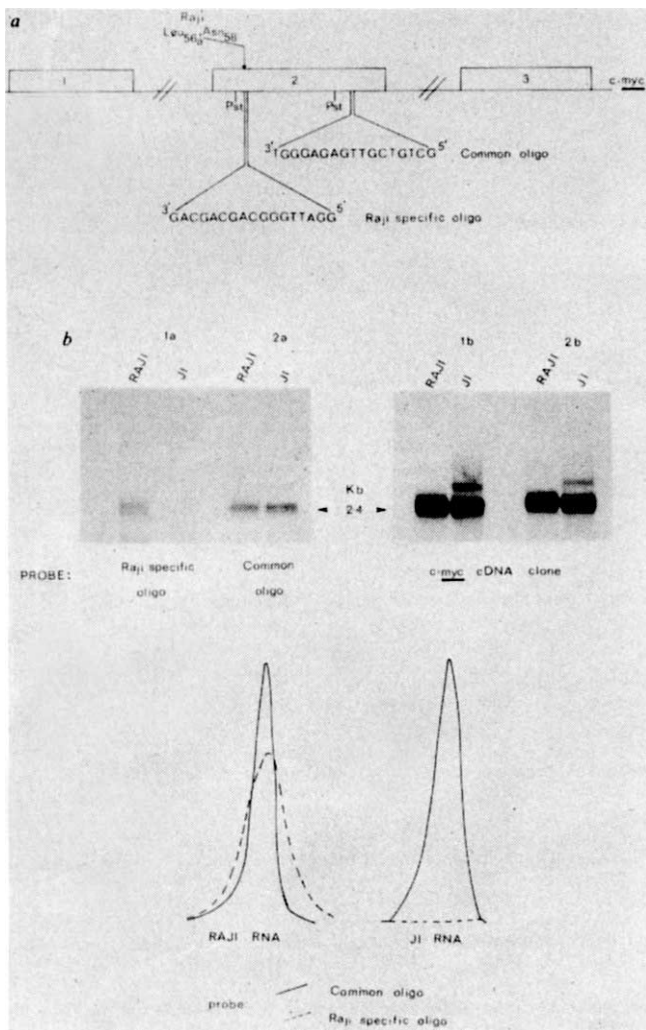


Fig. 5 Northern filter hybridization with *c-myc* oligonucleotide probes. **a**, Diagram of the location and sequence of the common *c-myc* complementary oligonucleotide (the same as used in Fig. 3) and the Raji translocated *c-myc* specific oligonucleotide. **b**, Autoradiographs of filter hybridizations using the various probes indicated. Two aliquots of 50 µg Raji and two of 10 µg JI poly(A)-containing RNA were glyoxylated⁴¹ and run in parallel on a 1.4% agarose gel, followed by electrophoresis and transfer to cellulose nitrate⁴¹ to yield two identical filters, each containing Raji and JI RNA. The filters were hybridized with ³²P-end-labelled oligonucleotides at 37 °C for 18 h in 2 × SSC containing 0.2% PVP, BSA, Ficoll³⁶, 0.1% SDS and 50 µg ml⁻¹ sonicated, denatured salmon sperm DNA. After hybridization the filters were washed at 37 °C with 2 × SSC, 0.1% SDS (six times for 10 min each), followed by autoradiography at -70 °C with prefogged film³⁷. When the autoradiograph had been obtained, both filters were rehybridized with a cloned *c-myc* probe, pUC cDIA¹⁸. The relative quantities of RNA in the autoradiographs were assessed by densitometry. **c**, Densitometer tracings from Raji and JI RNA hybridized with the two *c-myc* oligonucleotides.

densitometry; the densitometer tracings of the oligonucleotide hybridizations for JI and Raji mRNA are given in Fig. 5c. Taken at face value, the proportion of signal in *c-myc* mRNA from the translocated gene in Raji is about 80%. However, analysis of the intensities of the cloned *c-myc* hybridization suggested lower RNA levels in the Raji lane previously probed with specific oligonucleotide. Normalizing the data shows ~35% untranslocated and 65% translocated *c-myc* mRNA in Raji cells. Therefore, we conclude that both *c-myc* genes are transcribed in Raji cells, unlike other examples of Burkitt's lymphoma, and this unusual transcription of the normal allele correlates with the

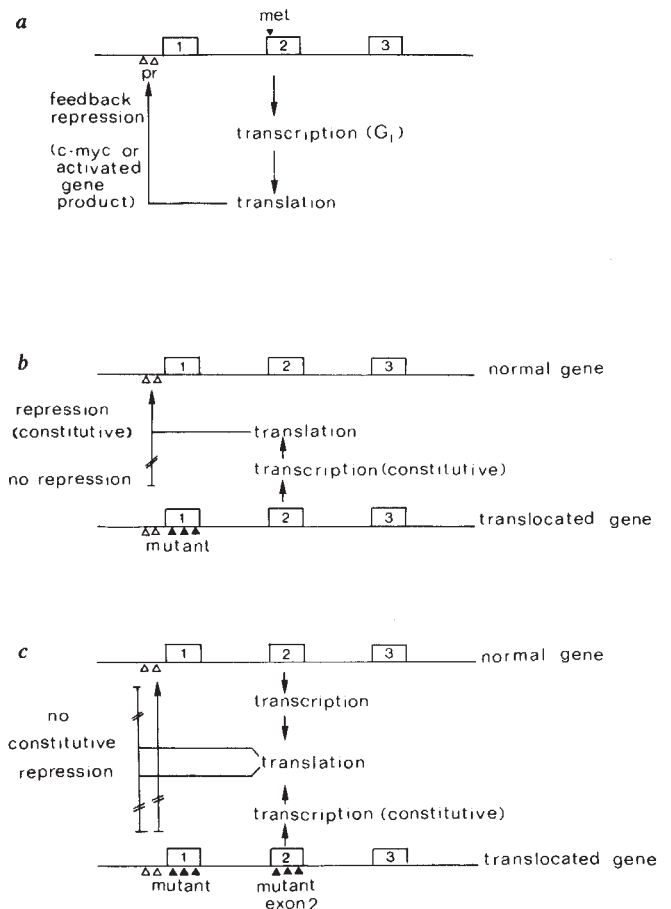


Fig. 6 Diagrammatic scheme of *c-myc* transcriptional control in normal cells (**a**) and Burkitt's lymphoma cell lines (**b**, LY67, Daudi; **c**, Raji). Note that the feedback repressor loop need not necessarily be via the *c-myc* protein itself but could occur via a gene activated by the *c-myc* protein.

presence of the codon replacements (that is, with protein alterations) in the translocated gene.

Immunological implications

In view of the data presented here, it now seems that the translocated *c-myc* very frequently suffers damage as a result of the chromosomal breakage. This is most dramatically demonstrated by studies of the gene in mouse myelomas where there is generally truncation of exon 1⁴; in Burkitt's lymphoma, where there is rarely loss of exon 1, there seems to be mutational damage. The difference between mouse and man in this respect is intriguing and may reflect the different rates of somatic mutation, which normally operate on the immunoglobulin variable-region genes, in these two organisms. Comparative hybridization studies of the immunoglobulin genes of mouse and man suggest that there may be as many as 10-fold more *V* genes in the former^{27,28}; it was deduced from this that the comparable *V* gene variability was created by an additional mutational load imposed on the *V* genes which rearrange to give active genes in man^{27,29}. The results concerning the translocation of *c-myc* support this notion and may explain why the loss of exon 1 is the more common activating event in mouse translocations. Conversely, in man mutational damage within the *c-myc* gene is the most common result, possibly because of the relatively high mutation rate in the B-cell population.

The results on the distribution of mutations and location of the BL translocated *c-myc* gene also have some implications for the mechanism of somatic mutation. Many of the translocations in man involve immunoglobulin *C_H* genes¹¹ and the breakage

occurs upstream of the *c-myc* gene (ref. 12 and see Fig. 1). Interestingly, the variant Burkitt's lymphomas break downstream of the *c-myc* gene and involve the proximity of the C_{λ} gene in t(8; 22)^{13,15,16} or apparently the C_{κ} gene in t(2; 8)³⁰, although the break has been reported to be near a V_H gene in one case¹⁴. These results suggest that the common requirement for somatic mutation may be the constant-region gene, either by enzymatic recognition of this sequence as suggested previously²² or perhaps by the particular chromatin configuration associated with the constant-region gene in these cells. In addition, it seems that the presence of an immunoglobulin constant-region gene near the translocation point is needed for the effect of the translocation to be exerted in the *c-myc* gene.

Model of *c-myc* gene activation

The data presented here show that there are substantial differences between translocated *c-myc* genes in Burkitt's lymphoma compared with normal sequences. These differences presumably arise from somatically introduced mutations associated with the DNA joining during chromosomal translocation and we would argue that the relatively restricted distribution of changes exists because such mutations provide a selective advantage (that is, growth advantage) on the affected cell. Although coding region changes do occur in Raji and LY67 (both within exon 2), as a general rule these are probably not important for oncogenesis (although they may have been in the case of Raji). More significant may be the fact that in the case of Raji, LY67 and JI, the sequence of exon 1 differs from the normal exon 1 sequence. As no coding function can be assigned to exon 1 of *c-myc*, it is possible that this segment, which is well conserved between mice and man^{7,12}, has a role in the control of transcription of *c-myc*²⁰. Our data on the alteration of the sequence of exon 1 support this idea by providing one explanation for the activation of the *c-myc* gene in at least some Burkitt's lymphoma lines even when the chromosomal breakpoint is at some distance from *c-myc*. Similar results have recently been obtained for other Burkitt's lymphoma translocated *c-myc* genes and a similar conclusion was reached³¹.

Furthermore, the results support a model in which transcription from the *c-myc* gene can be repressed by the binding of a protein to exon 1 (ref. 21), while explaining the maintenance of exon 1 with the translocated *c-myc* gene, without the requirement for elevated *c-myc* mRNA levels. Figure 6 shows this model,

which incorporates our data as well as the observation that the normal *c-myc* allele is usually inactive in cells carrying translocations^{12,23}. Figure 6a illustrates the normal transcriptional activity of the *c-myc* gene. In the resting cell, *c-myc* gene transcription is minimal but in response to stimulation (for example, mitogen³¹) transcription is initiated from promoter sites upstream of exon 1, followed by protein translation starting in exon 2. The *c-myc* protein product is then envisaged to function by activating a further set of genes (for example, those involved in DNA synthesis) which facilitate cell division. (This transcriptional role for the *c-myc* gene product seems a reasonable supposition as the protein seems to be related to the adenovirus gene E1A³² which itself displays transcriptional control properties³³.) At the same time, repression of *c-myc* gene activity begins by protein binding (either by direct binding of the *c-myc* protein itself or by a gene product activated by the *c-myc* protein) eventually resulting in the *c-myc* gene becoming quiescent again (in a cyclic fashion). In Burkitt's lymphoma (and mouse myeloma) translocation of the *c-myc* gene seems to subtly disrupt this cyclic transcriptional control. We must also consider the possibility that disruption of control of translocated *c-myc* genes occurs via immunoglobulin locus-activating elements which exert their effects on either exon 1 or some unknown regions upstream of the *c-myc* gene. As indicated in Fig. 6b, Burkitt's lymphoma cells (like LY67 and Daudi described here) exhibit constitutive transcription and translation of the translocated *c-myc* gene while at the same time constitutively repressing the normal *c-myc* allele (which, of course, has a normal repressor binding facility). The cell is therefore incapable of returning to the quiescent state of *c-myc* gene activity. The case of the Burkitt's lymphoma cell line Raji is an exception: here the normal allele is not repressed, which provides direct support for the theory that the *c-myc* protein is involved in transcriptional control as we know that the Raji translocated *c-myc* gene is abnormal²². Therefore, we envisage that these cells express both *c-myc* alleles (Fig. 6c) because mutations in exon 2 of the translocated gene product inactivate or significantly reduce its repressive function. The models predicted here and elsewhere^{21,31} are amenable to direct experimental testing, which is now being done using DNA transfer studies of manipulated *c-myc* genes in various types of lymphoid and non-lymphoid cell.

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Observational lower mass limit for black hole formation derived from massive X-ray binaries

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The high radial velocity amplitude (235 km s^{-1}) and 1.70-day orbital period of the B3V optical counterpart of the X-ray source LMC X-3 set a lower limit of $6\text{--}10 M_{\odot}$ for the mass of the X-ray source, strongly suggesting that it is a black hole^{1,2}. An observational upper limit to the mass of this compact star can be derived which, in combination with considerations about the evolutionary history of the system, allows us to derive an upper limit to the (zero age) mass of the progenitor of the compact star in this system: $\leq 80 \pm 10 M_{\odot}$. From the orbital parameters of pulsating massive X-ray binaries we find that stars with masses up to $\geq 40 (\pm 5) M_{\odot}$ (and, if supernova (SN) mass ejection is symmetric, up to $60 (\pm 7) M_{\odot}$) terminate life as neutron stars. Thus, if LMC X-3 is a black hole, the lower mass limit for terminating life as a black hole is in the range between $40 (60 M_{\odot})$ and $80 M_{\odot}$. Together with the initial mass function of massive stars this yields a black hole formation rate in the galaxy about two orders of magnitude smaller than the neutron-star formation rate. The direct progenitors of the black holes (just before the supernova) were massive Wolf-Rayet (WR) stars.

From the optical mass function of LMC X-3 ($2.3 M_{\odot}$), the absence of X-ray eclipses, in combination with the observed spectral type and absolute visual magnitude of the B-type companion, Paczynski² derived a lower limit to the mass of the B-star of $9.0 (\pm 6.0 M_{\odot})$, that is $\geq 3.0 M_{\odot}$. By comparing the observed absolute magnitude of this star with theoretical evolutionary tracks, he obtained an upper mass limit of $6.6 (\pm 2.0) M_{\odot}$, that is $\leq 8.6 M_{\odot}$. Thus, the allowed mass-range for the B-star is $3.0\text{--}8.6 M_{\odot}$. A lower limit to the mass M_H of the compact star is set by the absence of X-ray eclipses² which yields $M_H \geq 6.1$ and $\geq 10.0 M_{\odot}$, for $M_B = 3.0 M_{\odot}$ and $8.6 M_{\odot}$, respectively. Upper limits to M_H are set by the lower limit to the orbital inclination (i) derived from: (1) the observed rotational broadening of the spectral lines of the B-star, which yields $i \geq 50^\circ$ (ref. 1), and (2) the amplitude of the photometric variability of this star which, yields $i \geq 55^\circ$ (ref. 3). Adopting $i \geq 50^\circ$ as a safe lower limit we obtain $M_H \leq 9.0 M_{\odot}$ for $M_B = 3.0 M_{\odot}$ and $M_H \leq 13.6 M_{\odot}$ for $M_B = 8.6 M_{\odot}$. (For the shape of the allowed domain of M_B , M_H : see ref. 1.) The above derived largest and smallest M_B , M_H combinations are listed in the first two columns of Table 1. For a certain (M_B , M_H) combination one can derive an upper limit for the mass of the progenitor of the black hole just before its core collapse, as follows. Denoting the mass of the collapsed star before the SN as M_1 and assuming an initially circular orbit and instantaneous spherically symmetric ejection (that is, in a time interval shorter than the orbital period) of the SN shell (of mass $\Delta M = M_1 - M_H$) the post-SN orbital period, semi-major axis and orbital eccentricity are given by⁴

$$P_f = (\mu_f / (2\mu_f - 1))^{3/2} P_0; \quad a_f = a_0 \mu_f / (2\mu_f - 1);$$

$$e_f = (1 - \mu_f) / \mu_f \quad (1)$$

where subscripts zero and f denote the situation just before and after the SN, respectively, and $\mu_f = (M_H + M_B) / (M_1 + M_B)$ (we neglect the effects of the impact of a small fraction of the SN shell onto the B-star, as these as well as those of asymmetries are expected to be small^{5,6}).

As the present orbital period is only 1.70 days, tidal forces in the system are strong, and are expected to have rapidly circularized the orbit after the SN⁷. After tidal circularization

the semi-major axis has decreased by a factor $(1 - e_f^2)$ (we neglect the spin angular momentum of the B-star which is several orders of magnitude smaller than the orbital angular momentum). Using this, and applying Kepler's third law one finds that the orbital period P' and separation a' after tidal circularization are related to the pre-SN values by

$$P' = P_0 / \mu_f^2, \quad a' = a_0 / \mu_f \quad (2)$$

Because $P' = 1.70$ days, one thus can calculate P_0 for any assumed combination of M_B , M_1 and M_H . A strong constraint to the pre-SN mass M_1 of the compact star is set by the fact that, before the SN, the radius of the B-type star cannot have exceeded that of its Roche lobe, R_R , though must, on the other hand, at least have been as large as its zero age main sequence (ZAMS) value R_0 .

Using the expression for the Roche lobe given by Eggleton⁸, the condition that before the explosion R_0 was $\leq R_R$, in combination with the above equations yields the following condition for μ_f

$$\mu_f f^{2/3} / \{0.6 f^{2/3} + \ln(1 + f^{1/3})\} \geq \frac{R_0}{R_B} \left\{ \frac{q^{2/3}}{0.6 q^{2/3} + \ln(1 + q^{1/3})} \right\} \quad (3)$$

where $f = \mu_f / (1/q - \mu_f + 1)$, $q = M_B / M_H$ is the present mass ratio of the system, and R_B is the present radius of the B-star. R_B can be simply calculated, for the given M_B , M_H combination, from the fact that the B-star fills its Roche lobe in the present orbit.

For a given (M_B , M_H) combination, q and all quantities on the right-hand side are known, such that the lower limit on μ_f can be solved. The resulting limiting values of μ_f , M_1 , e and P_0 for the lower and upper limits of the range in (M_B , M_H) values are given in Table 1. For R_0 we used the values at an age of 3×10^6 yr—the approximate age at which the companion exploded—calculated by Van der Linden¹⁰ for $X = 0.70$, $Z = 0.01$ (the approximate metal abundance of the LMC). These are

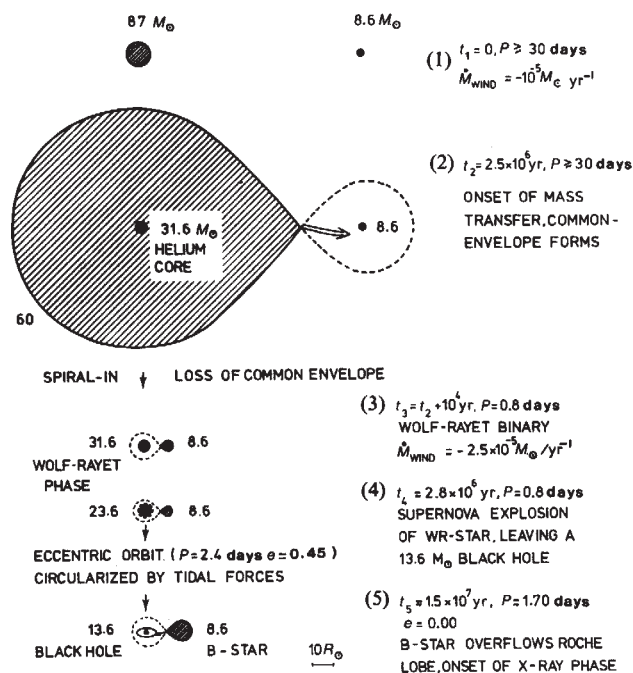


Fig. 1 Maximum-progenitor-mass evolutionary model for the origin of the LMC X-3 system, corresponding to the maximum possible masses of the compact star and its companion as allowed by the observations. Between stages (1) and (2) and between (3) and (4) the massive star undergoes stellar wind mass loss. Scale of (3), (4) and (5) is twice that of (1) and (2).

Table 1 Upper limits to the immediate pre-supernova mass M_1 of LMC X-3, and corresponding limits on μ_f , P_0 , a_0 , e , R_1 derived for the minimum and maximum possible values of the component masses M_B and M_H ; also shown are the range of helium-core masses and initial (zero-age) primary masses M_i corresponding to the M_1 values

	M_B ($M_\odot$)	M_H ($M_\odot$)	R_0 ($R_\odot$)	R_R ($R_\odot$)	μ_f $\geq$	M_1 ($M_\odot$) $\leq$	P_0 (day) $\geq$	a_0 ($R_\odot$) $\geq$	e $\leq$	R_1 ($R_\odot$)	M_{He} ($M_\odot$) $\leq$	M_i ($M_\odot$) $\leq$
Minimum	3.0	6.1	1.7	4.02	0.53	14.3	0.47	6.6	0.9	3.4–5.5	14.3–19.0	44.0–58.7
Maximum	8.6	13.6	3.47	5.75	0.69	23.6	0.81	11.7	0.45	5.5–7.2	23.6–31.5	69.4–86.9

Table 2 Lowest-progenitor mass evolutionary history for the 4U1223–62 system, taking minimum rates of mass loss by stellar wind and during the exchange stage into account, together with the evolutionary model for the 4U1223–62 system if the supernova mass ejection was spherically symmetric (to produce $e = 0.47$)

Lowest-progenitor mass evolutionary model for 4U1223–62 (asymmetric supernova)*			Evolutionary stage	Evolutionary model for 4U1223–62 with symmetric supernova†		
Approximate age (10^6 yr)	$M_1(M_\odot)$	$M_2(M_\odot)$		Approximate age (10^6 yr)	$M_1(M_\odot)$	$M_2(M_\odot)$
$t = 0$	42	38	(1) Zero age	0	66	34
3.9	34	31.2	(2) Onset of mass transfer	3.3	49	31
$3.9 + 10^4$ yr	15	44	(3) End of mass transfer	$3.3 + 10^4$ yr	28.5	45
4.35	10	43	(4) Supernova	3.59	22.7	44
4.35	1.4	43	(5) Post-supernova	3.59	1.4	44
5.2	1.4	40	(6) Present (X-ray phase)	5.0	1.4	40

* At the onset of the (case B) mass transfer the component masses have decreased to $34 M_\odot + 31.2 M_\odot$ due to stellar wind mass loss. Some $6 M_\odot$ is assumed to be lost during the exchange. During the $\sim 4.5 \times 10^5$ yr of helium burning and subsequent stages (between stages 3 and 4) the $15 M_\odot$ WR star is assumed to lose $5 M_\odot$, reducing its mass to $10 M_\odot$ at the time of the supernova. The $44 M_\odot$ companion needs about 1.3×10^6 yr after the mass exchange to terminate its hydrogen burning during which it loses $\sim 4 M_\odot$ by wind. This leads to the present system, consisting of a $\sim 1.4 M_\odot$ neutron star and a $40 M_\odot$ blue supergiant. In order to explain the observed orbital eccentricity ($e = 0.47$) the supernova mass ejection must have been very asymmetric in this model.

† Stellar wind mass loss and mass loss during the exchange was taken into account in the same way as in the other model (see text). If no mass loss occurs during the exchange, the zero age masses of the progenitor and its companion become $56 M_\odot$ and $33 M_\odot$, respectively.

listed in the third column. For any other (M_B , M_H) combination in the allowed range, the M_1 values are between those listed in Table 1. Notice from Table 1 that the lowest allowed value for μ_f is 0.53, which is consistent with the fact that disruption can have occurred only if $\mu_f \leq 0.50$ (ref. 9). The M_1 values in Table 1 are upper limits as we neglected the effects of impact, which would have further slightly enlarged e and P_f and thus would have lowered the maximum allowed M_1 values⁵.

The second step is to relate these M_1 values just before the SN, to the initial (zero age) mass of the progenitor. The very short orbital period and the large mass of the pre-SN star indicate that the pre-SN star must already have been compact (see the pre-SN Roche-lobe radii R_1 of the progenitor of the compact star in Table 1): a very evolved star of $\sim 20 M_\odot$ with a radius of only $\sim 5 R_\odot$ cannot have had any hydrogen envelope left, because such an envelope around a heavy-element core immediately expands to a large radius. Hence, the pre-SN star must have consisted of helium and heavier elements. A helium core of about $20 M_\odot$ requires an initial stellar mass of at least about $50 M_\odot$. Hence, the mass ratio of the initial zero age system was of the order of 0.1. In systems with initial mass ratios ≤ 0.3 – 0.4 the onset of mass transfer from the more massive (and thus more evolved) component to the less massive one leads to the formation of a common envelope in which the core of the massive star and the companion spiral in towards each other^{11–13}. Figure 1 (1)–(3) depicts this type of evolution schematically. The common envelope is lost on a short time scale ($\leq 10^4$ – 10^5 yr) as a consequence of large frictional heating^{13,14}. The very short present orbital period of LMC X-3 (and the even shorter pre-SN orbital period) clearly indicate that such spiral-in must have taken place here². (This evolution is the high-mass analogue of the formation mechanism of cataclysmic-variable binaries, see refs 12, 13.) After the spiral-in only the compact core of the massive star consisting of helium and possibly heavier elements, remained, with an orbital period of the order of a day or less. Stripped evolved cores of massive stars are thought to be iden-

tified with WR stars^{15,16}. Such stars have strong stellar winds, with mass loss rates $\dot{M} \sim (1\text{--}5) \times 10^{-5} M_\odot \text{ yr}^{-1}$ (refs 17, 18). During its lifetime of $\sim 3 \times 10^5$ yr (for $M_{He} \approx 25 M_\odot$) such a star may lose some $6 M_\odot$, so that the products of core-helium burning may become exposed at the surface, turning the star into a so-called WC star^{19,20}. The progenitor of the black hole in LMC X-3 just before the SN probably was such a WC star. The presence of helium in the spectra of WC stars shows that near the end of their nuclear evolution, when their heavy-element cores occupy 75% of their initial mass²¹, these stars have not yet been stripped entirely of their helium-rich outer layers. Hence, their mass at the time of the SN is ≥ 0.75 times the initial helium-core mass. This allows one to convert the pre-SN mass limits M_1 in Table 1 into upper limits on the helium-core mass of the progenitor. This yields the maximum M_{He} values in Table 1; the lower limits to M_{He} are equal to M_1 .

In turn, these M_{He} values can be converted into initial (zero age) stellar masses, by using the relation between initial stellar mass and helium-core mass, resulting from stellar-evolution calculations in which the effects of stellar wind mass loss during core-hydrogen burning are taken into account. The calculations by Chiosi *et al.*²² with mass loss parameter $\alpha = 0.83$, closely reproduce the observational estimates²³ of the amount of mass lost by massive stars during the core-hydrogen burning phase, and yield the relation

$$M_{He} = 0.1015 (M_i)^{1.285} \quad (4)$$

(for $X = 0.70$; for lower X values, M_{He} increases²⁴).

This yields the maximum values for the initial mass M_i given in Table 1, in the range 69.4 – $86.9 M_\odot$. Effects of convective overshooting, a smaller initial hydrogen abundance, and so on^{19,20,25,26}, all result in increasing the fractional mass of the helium core over that given by equation (4), and result in lower initial mass values for the progenitor of LMC X-3. We thus conclude that the upper limit to the mass of this progenitor is about $80 (\pm 10) M_\odot$. Figure 1 depicts the above described

evolutionary history of the LMC X-3 system for the maximum possible progenitor mass of $\sim 87 M_{\odot}$.

An estimate of the upper mass limit for stars that terminate their evolution as neutron stars can be derived from the presence of an X-ray pulsar in the most massive known X-ray binary system 4U1223-62. The X-ray mass function of this 41.4-day orbital period system is $31 M_{\odot}$ (refs 27-29). Together with the absence of X-ray eclipses—which sets an upper limit to the orbital inclination—this leads to a mass $\geq 40 M_{\odot}$ for the B1.5 Ia supergiant companion^{27,30}. To derive a lower limit for the mass of the progenitor of the neutron star in this system we have to consider evolution with mass exchange. To include the effects of stellar wind mass loss, we have again adopted the wind mass loss rates for hydrogen-burning O and B stars from ref. 22, as fitted by the parameter $\alpha = 0.83$. These rates are lower than given by most authors^{19,20} and are therefore suitable for deriving a lower mass limit for the progenitor. As to the mass loss from the system during the exchange: a comparison between the distributions of the mass ratios of the WR-binaries and their progenitors, the O-type spectroscopic binaries, shows that at least 30% of the envelope of the primary star is lost during the exchange^{32,33}. Using the condition that we wish to avoid, tandem evolution (see ref. 37), we find that the lowest-mass realistic progenitor system of 4U1223-62 has components of $42 M_{\odot}$ and $38 M_{\odot}$. Table 2 lists the component masses at various evolutionary stages. As we have adopted the lowest possible mass loss rates compatible with the observations (both by wind and during the exchange) it seems that $42 M_{\odot}$ is about the lowest possible progenitor mass for the neutron star. If no mass loss during the exchange were adopted (but wind mass loss still is included) the smallest-mass progenitor system would be $37 + 33 M_{\odot}$. Taking uncertainties in, for example, wind mass loss rates and overshooting, into account—the resulting lower limit to the mass of the progenitor of the pulsar 4U1223-62 is $40 \pm 5 M_{\odot}$.

With the progenitor system of Table 2 it is impossible to explain the observed orbital eccentricity $e = 0.47$ of the 4U1223-62 system unless the supernova mass ejection was highly asymmetric. For spherically symmetric ejection the eccentricity expected from equation (1) is only 0.19. (Due to the extensive mass exchange the pre-SN orbit is expected to have been circular.) If the SN mass ejection is assumed to have been spherically symmetric, the observed eccentricity $e = 0.47$ together with equation (1) yields a mass of $22.7 M_{\odot}$ for the progenitor of the neutron star at the time of the explosion. The evolutionary history of the system for this case is listed in Table 2 and the corresponding zero age mass of the progenitor seems to be about $60 (\pm 7) M_{\odot}$.

Combining the results of Tables 1 and 2 we conclude that the lower (zero age) mass limit for stars which terminate their evolution as a black hole is in the range between $40(\pm 5) M_{\odot}$ and $80 (\pm 10) M_{\odot}$. If SN mass ejection is spherically symmetric this limit is between $60 (\pm 7)$ and $80 (\pm 10) M_{\odot}$. We expect these limits also to be good approximations for single stars, as single stars with masses $\geq 40 M_{\odot}$ have very large rates of wind mass loss and are expected to turn into WR stars and evolve in a way similar to that of binary components^{15,19,20}. Knowledge of this limit is of great importance for estimating the bulk yield of nucleosynthesis by massive stars²¹.

According to Garmany *et al.*³⁴ the number of O and WR stars originating from stars more massive than $40 M_{\odot}$, $60 M_{\odot}$ and $80 M_{\odot}$ within 2.5 kpc from the Sun is between 100 and 132, between 25 and 55, and between 8 and 10, respectively.

Assuming mean lifetimes of 2.5×10^6 yr, 3×10^6 yr and 3.5×10^6 yr for stars more massive than $80 M_{\odot}$, $60 M_{\odot}$, and $40 M_{\odot}$ respectively, this yields a black hole formation rate in the solar neighbourhood in the galactic plane of $(0.2, 0.9 \text{ and } 2.0) \times 10^{-6} \text{ kpc}^{-2} \text{ yr}^{-1}$, respectively. This is about two orders of magnitude smaller than the galactic SN rate³⁵ and the pulsar birth rate³⁶.

Finally, because about half of all black holes are expected to be formed in binaries, a better knowledge of the number of

black hole X-ray binaries in the galaxy, in combination with the initial mass function, will allow an independent estimate of the lower mass limit for black hole formation.

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Discovery of M87-like ejection in F-71

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The M87 jet^{1,2} is one of the most obvious manifestations of violent activity inside the compact nuclei of galaxies whilst also being one of the most spectacular phenomena of extragalactic astronomy. Although many radio jets are known, very few optical jets have been detected and virtually none is comparable to the prototype jet in M87. Recent charge-coupled device (CCD) images of the southern object F-71 show that this elliptical galaxy ($z = 0.057$) exhibits a brilliant knot ~ 14 arc s away from the nucleus and is physically connected to it. Optically, this knot resembles in many respects the bright knots A and B in the M87 jet: its spectrum is featureless and very blue with a slope ($\alpha = 1.7 \pm 0.1$) equal to that of the M87 knots. Its relative luminosity with respect to, and its separation from, the nucleus are both greater than in the M87 galaxy-jet system. We suggest here that the most likely interpretation of the F-71 knot is that it is ejected from the parent galaxy in the same way as the knots in the M87 jet. We propose that further observations of the F-71 system might provide important clues to our understanding of the ejection activity from the nuclei of galaxies.

The object F-71 ($\alpha_{1950} = 20^{\text{h}} 14.8^{\text{m}}$, $\delta_{1950} = -57^{\circ} 43'$) from Fairall's list³ of compact galaxies appears as an almost circular galaxy of approximate magnitude 16 mag on the European

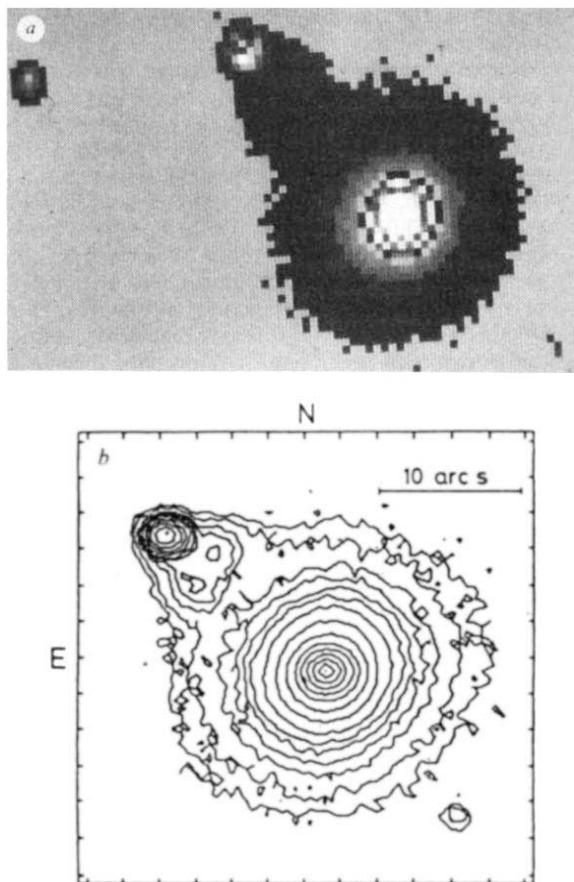


Fig. 1 *a*, CCD image in the *r*-band of F-71 showing the connection between the nucleus of the elliptical galaxy and its nonthermal knot. *b*, Contour plot of the CCD image in the *r*-band of F-71.

Southern Observatory (ESO) Quick Blue Survey plate. Towards the north-east of the object a starlike companion is visible approximately 10 arc s away which has not been described before. CCD images of F-71 have been obtained on 7 and 8 May 1983 with the 1.5-m Danish telescope at La Silla (exposure time 45 min, seeing 1–2.5 arc s, 1 pixel ≈ 0.471 arc s) through various filters (*B*, *V*, *r*, *z*). All these images reveal a physical connection between the north-east knot and the parent galaxy.

As an example, we display our observational results for the *r*-band: the CCD direct image (Fig. 1*a*) shows the connection between the knot and the nucleus. The isophote representation (Fig. 1*b*) and the intensity tracing through the nucleus and the knot (Fig. 2) reveal the light distribution in more detail. The brightness distribution of the parent galaxy closely follows a de Vaucouleurs law, and is classified as an elliptical galaxy of type E0–E1. Its minor axis points in the direction of the optical north-east knot, which is located $13.75'' \pm 0.3''$ away from the galaxy centre in position angle (PA) $49.5^\circ \pm 0.5^\circ$. A possible weak 'counter-jet' is indicated in the direction $221.5^\circ \pm 0.5^\circ$ and $12.65'' \pm 0.3''$ away from the galaxy centre, and shows up particularly well in the red bands *r* and *z*. The north-east knot appears slightly extended on the far side compared with stellar images on the same CCD-frames. On the near side, the optical isophotes in all filter bands show a backward extension of the compact north-east knot nearly symmetrically with respect to the line joining the nucleus and the knot (see Fig. 1 for the *r*-band). This feature is reminiscent of the "waste energy baskets" described by Longair *et al.*⁴ in connection with the radio hotspots of extended radio galaxies, in particular of Cyg A. The detection of this tailed structure in all filter bands supports a physical connection between the blue knot and the galaxy as opposed to the possibility of a chance superposition of F-71

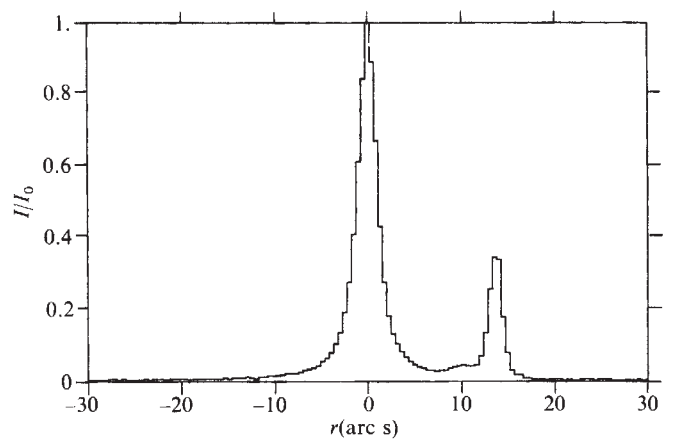


Fig. 2 Tracing through the nucleus of F-71 and the north-east knot in the *r*-band.

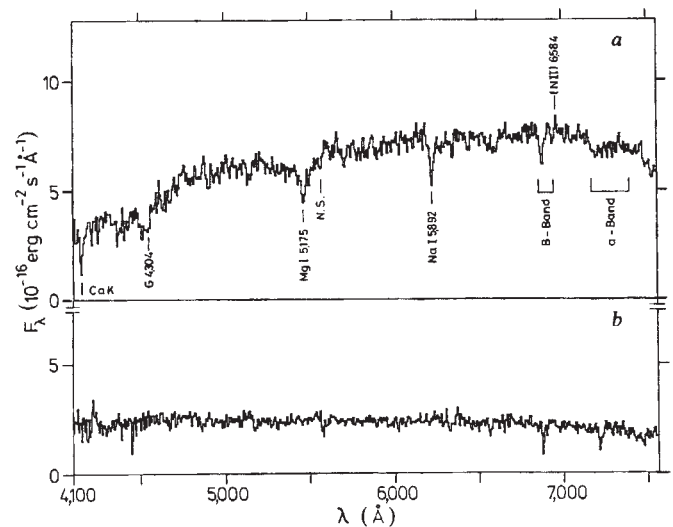


Fig. 3 The spectra of the nucleus of F-71 (*a*) and of the north-east knot (*b*).

and a background object. From the tracings through the galaxy and the north-east knot we derive relative peak intensities of the knot with respect to the galaxy centre for the *z*, *r*, *V* and *B* bands, increasing towards the blue as follows: 0.31, 0.36, 0.37 and 0.53.

Optical spectra of both the galaxy and the north-east knot of F-71 were obtained on 2 September 1983 at the ESO 3.6-m telescope equipped with the Boller and Chivens spectrograph and the IDS (entrance aperture $4'' \times 4''$, dispersion $171 \text{ \AA mm}^{-1}$, wavelength range 4,100–7,300 $\text{\AA}$, exposure time 20 min each). The galaxy spectrum (Fig. 3*a*) shows absorption lines typical of elliptical galaxies. Fairall suspected, on the basis of his low-dispersion slitless spectrum of F-71, the presence of Balmer emission features. From our spectrum we cannot confirm this conjecture. $H\alpha$ is coincident with the atmospheric B-band. There is marginal evidence for the presence of weak [N II] 6,584 emission. From the absorption lines we derived a redshift $z = 0.0573$ which puts F-71 at a distance 229 Mpc ($H_0 = 75 \text{ km s}^{-1} \text{ Mpc}^{-1}$). The visual apparent and absolute magnitudes derived from the flux at 5,500 $\text{\AA}$ are $m_V = 16.8 \text{ mag}$, and $M_V = -20.0 \text{ mag}$. With a scale $1.1 \text{ kpc arc s}^{-1}$ the north-east knot is at a projected distance 15.3 kpc from the galaxy centre.

The optical spectrum of the knot appears to be featureless and flat (see Fig. 3*b*) but does not entirely rule out the possibility that the spikes in the short wavelength end of the spectrum at $\lambda 4,216 \text{ \AA}$ and $\lambda 4,453 \text{ \AA}$ are real and are [O II] $\lambda 3,727$ and

CaK λ 3,934 of a BL Lac object at $z=0.13$. However, in view of the morphological evidence described above and shown in Figs 1 and 2, this interpretation does not seem to be tenable. The mean spectral index of the optical continuum of the knot is $\alpha = 1.7 \pm 0.1$ ($F_\nu \propto \nu^{-\alpha}$). The tracings through the nucleus and the knot of F-71 show that in all the colour bands the relative brightness and concentration of light between the jet and nucleus is very similar to those in M87 and its jet¹. This may be taken as further evidence that both phenomena have the same physical origin.

F-71 and its north-east knot resemble in many respects the situation of the giant elliptical galaxy M87 with its spectacular nonthermal jet. Like M87, F-71 is an elliptical galaxy of the type E0–E1. In M87 the one-sided jet is made up of several nonthermal knots, while in F-71 a single strong knot is visible to one side and a weak feature to the opposite side. The flat and apparently lineless spectrum of the north-east knot corresponds to the nonthermal spectrum of the knots A and B of M87 displayed by Sulentic *et al.*². Intriguingly, the spectral slope $\alpha = 1.7 \pm 0.1$ of the F-71 knot continuum matches exactly that of the knots A and B in the M87 jet, $\alpha = 1.7 \pm 0.2$ (ref. 5). Intrinsically, the scale of the F-71 phenomenon is an order of magnitude larger than in the M87 prototype. In F-71 the north-east knot is much more clearly separated from the main body of the galaxy than the jet in M87, and its luminosity, relative to the galaxy, is much stronger.

The optical data on F-71 described here characterize this source as a galaxy with similar ejection activity as M87, thus warranting further extensive studies, in particular in the radio region.

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Conjugate ionospheric flows on Uranus

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Consideration of the pointing direction and rotation period of the spin of Uranus suggests that a novel kind of day-to-night ionospheric flow system may exist there. For equatorial distances $r < r_c$ ($\approx 2.74 r_p$), the conjugate ionospheric flow from the dayside hemisphere to the nightside hemisphere could become supersonic as it crosses the equator and a shock forms at the termination point; for $r > r_c$, smooth transition at the contact point between the ionospheric flow from the dayside hemisphere and the nightside atmosphere is possible. The rotational effect of Uranus also limits the inner extension of the thermal plasma disk generated from energetic particle sputtering of the ring-particles and satellites. The combined effect of the dynamic transport of the ionospheric plasma and the injection of ring plasma into the upper atmosphere could provide interesting results concerning the ionosphere of Uranus, which is to be explored by the Voyager 2 spacecraft in 1986.

As a result of the slow rotation of Venus, its nightside atmosphere is not subjected to a photoionization effect; however, spaceprobes to Venus have found that it has a very dynamic and sizeable nightside ionosphere^{1,2}. Its maintenance has been suggested to result from impact ionization by energetic electrons

(100 eV–1 keV) accelerated in the magnetic tail^{3,4}, or from transport of ionospheric plasma from the dayside to the nightside^{1,5}. The ionospheric transport process of Venus, driven by solar heating and solar wind interaction directly with the dayside atmosphere, is unique among the planetary ionospheres. Note that a similar day-to-night ionospheric flow pattern could also occur at Uranus because although Uranus has a rapid spin period (16.31 ± 0.27 h; see ref. 6) its rotational axis is pointing towards the Sun. This means that while one hemisphere is illuminated constantly by sunlight the other is always in darkness. Therefore, only the dayside atmosphere is affected by photoionization—just as for Venus. One major difference between Venus and Uranus, however, is that while Venus has little or no intrinsic magnetic field, Uranus should possess an intrinsic magnetic field of significant strength as recently indicated by the UV observations of enhanced H Ly α emissions at Uranus^{7–9}. In fact, Hill *et al.*¹⁰ have postulated that the equatorial surface dipole field should be ≈ 4 G.

The expected hemispherical flows in the closed field-line region are shown in Fig. 1. In the simplest situation, the photoelectron–H⁺ flux streaming out of the dayside hemisphere along a flux tube will cross the equator and then be absorbed at the conjugate point.

Rösler¹¹ has discussed the presence of such conjugate-point ionospheric flows at the terrestrial ionosphere during solstices at high altitudes. The occurrence of this type of inter-hemispheric flow should be much more widespread at Uranus as a result of the peculiar orientation of its rotational axis at present. Following the standard treatment in solar wind theory, the hydrodynamic equations describing the ionospheric flows (assumed to be isothermal) can be combined into¹²:

$$\frac{1}{2}(M^2 - M_0^2) - \ln\left(\frac{M}{M_0}\right) - \ln\left(\frac{A}{A_0}\right) + \frac{\phi - \phi_0}{c_0^2} = 0 \quad (1)$$

where $M = u/c$ is the ratio of the flow speed to the sound speed given as $c^2 = 2kT/m$ (kT is the plasma temperature, and m the proton mass), and A is the cross-sectional area of the flux tube. Also we have

$$\phi_g = -\frac{GM}{r} \quad (2)$$

$$\phi_c = -\frac{\Omega^2 r^2 \cos^2 \theta}{2} \quad (3)$$

where G is the gravitational constant, M the planetary mass ($= 8.7 \times 10^{28}$ g), r the radial distance, θ the latitudinal angle, and Ω the angular rotation frequency ($\Omega = 1.07 \times 10^{-4}$ rad s⁻¹). In

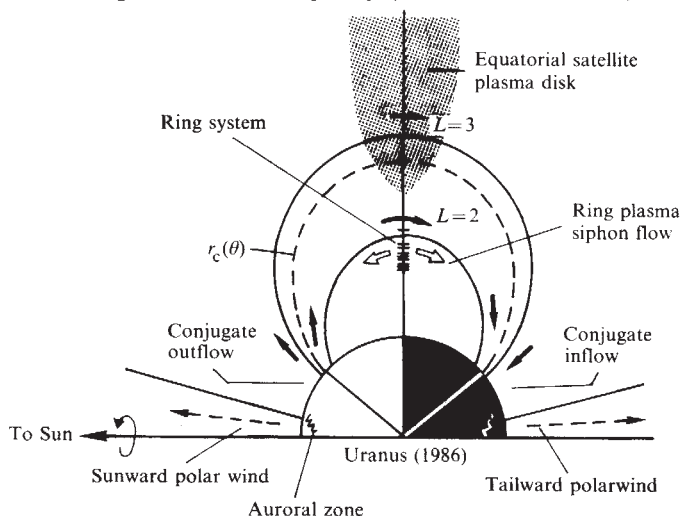
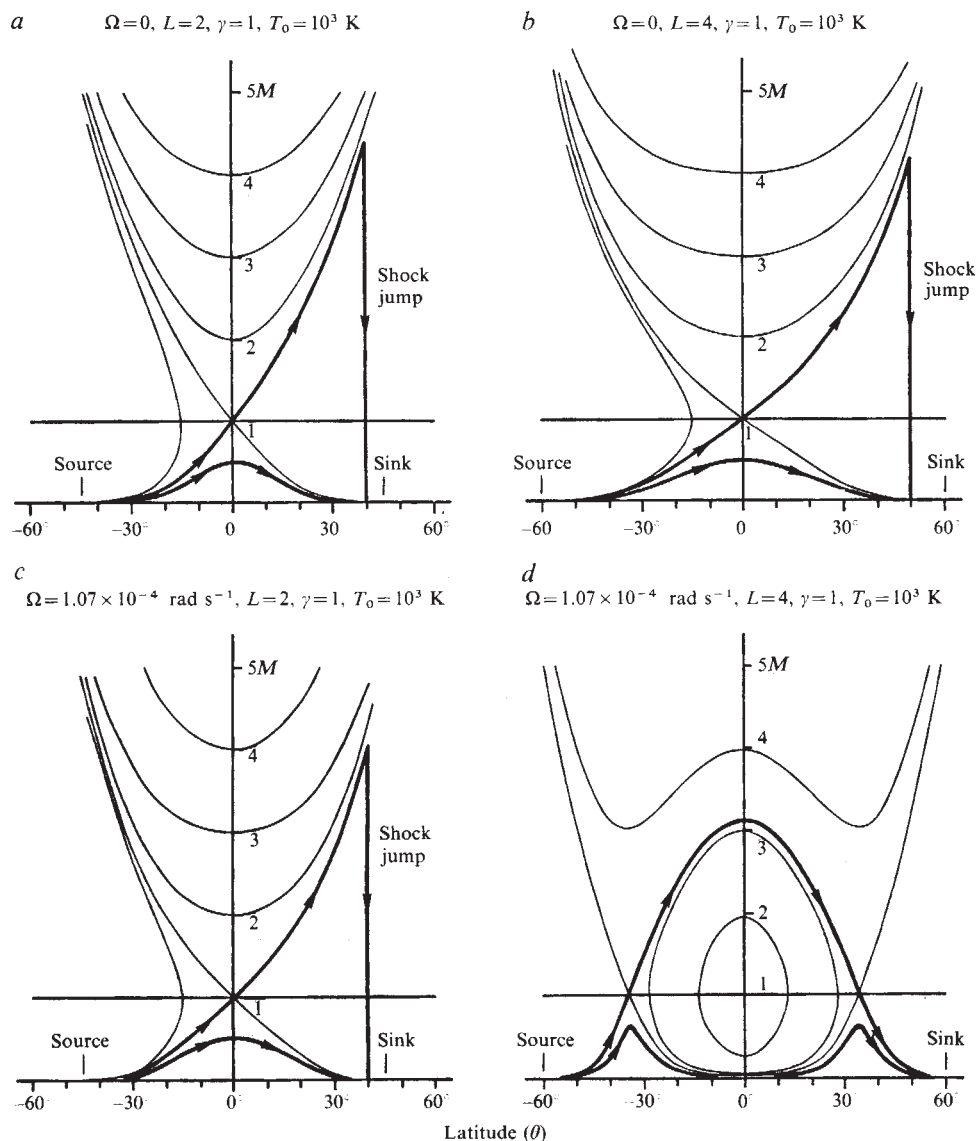


Fig. 1 The conjugate ionospheric flow pattern at Uranus. Such inter-hemispheric flows could coexist with a siphon flow of thermal plasma originates from the equatorial region as a result of energetic charged sputtering, meteoroid bombardment, and photosputtering of the rings and satellites.

Fig. 2 Examples of isothermal conjugate ionospheric flows at Uranus with the plasma temperature set at $T = 1,000$ K. Four cases are considered: *a*, non-rotating case (angular frequency $\Omega = 0$) and the field-aligned flow crosses the equator at a radial distance of $2.0r_p$ ($L = 2.0$); *b*, $\Omega = 0$ and $L = 4.0$; *c*, $\Omega = 1.07 \times 10^{-4}$ rad s $^{-1}$ (rotation period = 16.31 h) and $L = 2.0$; *d*, $\Omega = 1.07 \times 10^{-4}$ rad s $^{-1}$ and $L = 4.0$.



equation (1) $\phi = \phi_g + \phi_c$ and the subscript (0) denotes values corresponding to radial distance r_0 and latitude θ_0 ($r = r_c \cos^2 \theta$ where r_c is the radial distance at the equator).

In the case of a non-rotating planet, several solutions to equation (1) are possible. These include: (1) transition from subsonic flow to supersonic flow before reaching the equator and then going through the reverse transition at the corresponding conjugate point; (2) reaching the sonic point at the equator and then retaining supersonic flow speed all the way to the other hemisphere; (3) retaining subsonic speed at all positions.

All of these solutions could be generated by adopting different sets of pertinent parameters (such as initial flow speed and plasma temperature) for the hydrodynamic flows. A more complete description of them will be presented elsewhere. We have depicted only a subset in Fig. 2 to demonstrate the idea of a conjugate ionospheric flow as well as the potential importance of the planetary rotation in modifying such flows. Figure 2 shows how the ionospheric flow from the dayside hemisphere might go through a sonic point as it crosses the equator or it might remain subsonic all the way to the nightside hemisphere if it starts with a smaller Mach number. In the supersonic flow case, a shock forms at the termination point as the ionospheric plasma slams into the darkside atmosphere. Such flow patterns hold for a wide range of radial distances as shown in Fig. 2a, b. However, for Uranus, there is an important modification to the flow dynamics at large radial distances; this is because the rapid rotation of the planet means that centrifugal effects begin to have a role in the force balance beyond a certain point. In the

simplest possible situation, let us consider first the motion of one single charge particle. In a cold plasma approximation, neglecting the magnetic moment, the balance^{13,14} between gravitational and centrifugal accelerations at the equator will define a radial distance (in the case of a perfect dipole field)

$$r_c(\theta) = \left[\frac{2GM \sec^2 \theta}{3\Omega^2} \right]^{1/3} \quad (4)$$

such that the charged particles with zero magnetic moment will be kept to the equator when $r > r_c$ but will be pulled to the topside atmosphere when $r < r_c$. Because $r_c(\theta = 0) = 6.96 \times 10^4$ km ($= 2.74r_p$) we have $\theta_c = 53^\circ$.

In the case of the ionospheric flow, a similar limit could apply. Figure 2 provides the most extreme examples of the modification effect by the centrifugal force on the conjugate ionospheric flow. Briefly, for flows with sonic point at the equator, those originating from latitudes $\theta < \theta_c$, the 'X-point' configuration near the equator remains unchanged. On the other hand, for those originating from latitudes $\theta > \theta_c$, the corresponding X-point configuration could be substituted by the O-point configuration such that the plasma flows should be subsonic as they reach the other hemisphere (see Fig. 2c, d). Note that the general nature of an O-type flow in a non-rotating planet remains unchanged if the rotational motion of the planet is introduced. In addition to these many possible variations in the flow patterns, the net results are that the dayside upper ionosphere should be constantly in a state of dynamic expansion instead of diffusive

equilibrium, and that the nightside atmosphere will receive a supply of ionization in this way, and a nightside ionosphere may be maintained accordingly.

In the case of Saturn, it has been postulated that the ionospheric region with magnetic field lines touching the rings should also approach the condition of free streaming—as a result of the absorption effect of the rings¹⁵. Because of the narrowness of the rings of Uranus, they would be less efficient in depleting the ambient plasma density in the vicinity of the ring system. The ϵ ring with a width of 50–100 km, however, should be able to intercept the ionospheric plasma and convert the hydrogen ions to neutralized H atoms. The ϵ ring should also act as an absorbing barrier in keeping the magnetospheric energetic charged particles from diffusing inward further than $2.4r_p$ (uranian radius). Cheng and Lanzerotti¹⁶ have suggested that energetic particle sputtering of the rings could be an important cause of the thermal plasma in the magnetosphere. The associated production of the sputtered neutrals will generate a thin neutral cloud, reflecting the composition of the ring particles. The subsequent ionization of these heavy neutrals (that is, CH_4 , H_2O and their fragments) will form a plasma disk at the equator. Force balance relation as considered in previous studies^{14,17} indicates that the thermal plasma disk (if it exists) could be essentially limited to radial distances outside r_c . In other words, as shown in Fig. 1, a 'siphon' plasma flow from the equatorial region to the upper atmosphere should be expected in addition to the conjugate ionospheric flow. The dynamic transport of ionization from one hemisphere to another and the simultaneous loading of the atmosphere with hydrocarbons from the rings could have rather interesting effects on the ionospheric system of Uranus.

Finally, questions may be raised about how the presence of a plasma torus might affect the conjugate flow pattern. The most obvious effect is that the angular velocity of the magnetospheric plasma may be reduced to a value smaller than what is expected of rigid co-rotation, and the corresponding centrifugal force may be less important. In addition, the magnetic field configuration might be significantly changed from that of a dipole field.

Two mechanisms could be relevant here. First, as discussed previously¹⁸ for the case of a rapidly-rotating magnetosphere^{18,19}, if the plasma density is large enough such that at a certain point the kinetic energy of the co-rotation motion exceeds that of the (dipolar) magnetic field energy, namely $\rho V^2/2 \geq B^2/8\pi$, no rigid rotation will be enforced; instead a radial outflow of the magnetospheric plasma stretching the magnetic field outward is expected to occur beyond this critical point. For Uranus, to reach this condition at $L \approx 4$ requires the plasma number density (assumed to be O^+ ions) to exceed $n_c \approx 10^7 \text{ cm}^{-3}$ if the equatorial surface field $B_0 \approx 4 \text{ G}$, and $n_c \approx 4 \times 10^4 \text{ cm}^{-3}$ at $L \approx 8$ (the critical values of n_c are a factor of 100 smaller if $B_0 \approx 0.4 \text{ G}$). But as shown by the Voyager plasma experiments²⁰ and theoretically demonstrated by Hill²¹, in the jovian magnetosphere, non-rigid co-rotation could take place even with much less magnetospheric plasma—simply as a result of angular momentum transfer from the planetary ionosphere to the Io torus plasma. However, a large degree of non-rigid co-rotation ($\Delta\Omega/\Omega \sim 50\%$) only takes place at large distances from the source region of the thermal plasma. In the vicinity of the source region, it is the mechanism of pick-up current which has more immediate effect on the rotational rate of the local magnetospheric plasma.

Following the arguments given in refs 22–24, it can be shown that a reduction of the angular velocity of the magnetosphere will be necessitated by the acceleration of the new ions of satellite or ring origin by a pick-up current system. If the total production rate of the ions is $\dot{M}$, the radial dimension of the source region ΔR , and the integrated ionospheric Pedersen conductivity Σ_p , we have

$$\frac{\Delta\Omega}{\Omega} \approx \frac{\dot{M}L^5}{4\pi B_0^2 \Sigma_p R_0 \Delta R} \quad (5)$$

For Jupiter, $\dot{M} \approx 1.5 \times 10^4 \text{ kg s}^{-1}$, $L \approx 6$, $\Delta R \approx R_0 (= 7 \times 10^7 \text{ m})$

and $\Sigma_p \approx 0.2 \text{ S}$, we have $\Delta\Omega/\Omega \approx 6\%$ which is in approximate agreement with observations^{25,26}. By comparison, for Uranus with $\dot{M}$ assumed to be $\leq 10^2 \text{ kg s}^{-1}$, $L \approx 4$, $\Delta R \approx R_0 (= 2.5 \times 10^7 \text{ m})$ and $\Sigma_p \approx 0.01 \text{ S}$ we have $\Delta\Omega/\Omega \leq 1\%$. Therefore, we expect the plasma disk of Uranus within $L \approx 5$ to be rigidly co-rotating and the magnetic field topology to be hardly affected by the mass loading effect of the pick up ions (if $\dot{M} \leq 10^2 \text{ kg s}^{-1}$). For larger values of L (≥ 10), it is more difficult to assess the situation as there are many uncertainties. In any case, one crucial parameter in this problem is the integrated ionospheric Pedersen conductivity Σ_p which is directly related to the whole process of transport of ionization from the dayside ionosphere to the nightside hemisphere.

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Silicon and aluminium site distributions in 2:1 layered silicate clays

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Although the layer charge in 2:1 phyllosilicate minerals is known to result from the replacement of tetrahedral Si or octahedral Al, Fe and Mg by ions of lower charge, there is only limited information concerning the distribution of layer charge from X-ray crystallographic data^{1–3}. Here we use ²⁹Si and ²⁷Al magic angle spinning (MAS) NMR spectroscopy to examine the site distribution of tetrahedral Si and Al in a series of synthetic trioctahedral clays with (Si/Al)_{tetr} values in the range 2.74–7.69. Analysis of the ²⁹Si spectra shows that Loewenstein's rule for Al occupancy of the tetrahedral sheet is obeyed and that there is some short-range ordering for Al sites. The nature of the ordering is explained in part by the results of electrostatic potential energy calculations. In general, strong ²⁷Al resonances are observed for Al in tetrahedral sites, but the resonances due to Al in octahedral sites are considerably weaker than would be expected on the basis of chemical analysis. Consequently, no quantitative analysis of the ²⁷Al spectra is possible.

Table 1 Unit cell compositions, cation exchange capacities and X-ray diffraction data for synthetic 2:1 layered silicate clays

Compound	Unit cell composition*	(Si/Al) _{tetr}	CEC, mequiv. per 100 g†		d ₀₀₁ (Å)‡	
			Calculated	Observed	Air-dry	Glycerol solvated
(I)	Na _{0.68} [Mg _{5.80} Al _{0.20}](Si _{7.08} Al _{0.92})O ₂₀ (OH) ₄	7.69 ± 0.23	88	91	13.1	18.0
(II)	Na _{0.93} [Mg _{5.93} Al _{0.19}](Si _{6.64} Al _{1.36})O ₂₀ (OH) ₄	4.88 ± 0.08	119	134	14.2	14.6
(III)	Na _{1.26} [Mg _{5.45} Al _{0.44}](Si _{6.51} Al _{1.49})O ₂₀ (OH) ₄	4.37 ± 0.12	161	170	14.0	14.5
(IV)	Na _{1.60} [Mg _{6.17} Al _{0.08}](Si _{5.86} Al _{2.14})O ₂₀ (OH) ₄	2.74 ± 0.04	201	201	14.2	14.9

* The reported unit cell compositions and (Si/Al)_{tetr} values are averages of five independent analytical determinations using inductively-coupled plasma emission spectroscopy. Samples were fused in lithium borate before analysis, and NBS plastic clay no. 98a was used as an analytical standard.

† Cation exchange capacities (CEC) were obtained by NH₄⁺ saturated and subsequent determination of Kjeldahl nitrogen.

‡ X-ray spacings were determined for oriented film samples using CuKα radiation.

Loewenstein's rule⁴ states that during the isomorphous substitution of Si by Al, occupancy of adjacent tetrahedral sites by Al is avoided. An early X-ray crystallographic study¹ of Si-Al ordering in the archetypal 2:1 mica muscovite has led to a model in which the Al centres are arranged in chains, clearly violating Loewenstein's rule. More recent crystallographic studies^{2,3} provide evidence for long-range ordering and separation of Al centres in the brittle mica margarite, but not in muscovite. The lack of longer-range order for muscovite does not preclude the possibility of tetrahedral Al ordering within domains, preserving Loewenstein's rule, but such short-range order is difficult to demonstrate crystallographically. Thus experiments which provide incisive information on tetrahedral site distributions are needed for the elucidation of 2:1 phyllosilicate structures.

Recent MAS-NMR studies of zeolites have shown the ²⁹Si chemical shifts to be sensitive to the number of Al ions occupying near-neighbour tetrahedral sites^{5,6}. Moreover, the relative signal intensities can provide structural information which cannot be obtained by X-ray crystallography⁷. ²⁷Al MAS-NMR at high field strengths offers the possibility of providing structural information in some systems⁷⁻⁹. MAS-NMR can be equally useful for addressing the question of tetrahedral site ordering in phyllosilicates, provided that the concentration of structural paramagnetic ions is not large.

Trioctahedral clays of suitable composition and layer charge were synthesized by the hydrothermal crystallization of gels¹⁰ at 300 °C and 100 bar over a period of 4 weeks. Table 1 provides the chemical composition and pertinent physical properties for each clay sample. Sample (I), which undergoes swelling in glycerol, may be described as a synthetic analogue of Na⁺-saponite. Samples (II) and (III) carry a layer charge near the upper limit for a saponite, but they do not form a glycerol complex. Sample (IV) does not intercalate glycerol, but the layer charge is closer to the value expected for a vermiculite or mica.

Typical ²⁹Si MAS-NMR spectra are shown in Fig. 1a for samples (I), (III) and (IV). The relationship between the relative intensities of the ²⁹Si resonances and the (Si/Al)_{tetr} values allows us to assign the lines from highest to lowest field to silicon centres in which the aluminium occupancy of the three near-neighbour tetrahedral sites is (0 Al), (1 Al) and (2 Al), respectively. None of the clays exhibits a resonance which can be attributed to Si with a near-neighbour site occupancy of (3 Al). The average chemical shifts are -95.1 ± 1.1 (0 Al), -90.1 ± 0.9 (1 Al), and -85.5 ± 0.8 p.p.m. (2 Al). It seems clear that the shifts depend mainly on the near-neighbour tetrahedral site occupancy. No second nearest-neighbour effects are observed, and there is little or no dependence of the shifts on layer charge or aluminium content of the octahedral sheet.

Representative ²⁷Al MAS-NMR spectra are illustrated in Fig. 1b. All of the samples exhibit an intense ²⁷Al resonance near 65 p.p.m. which is indicative of Al in a tetrahedral site⁷. Some samples (such as (IV)) also exhibit an unresolved tetrahedral Al component upfield of this resonance. Aluminium in an octahedral environment is clearly visible as a weak signal near 5 p.p.m.

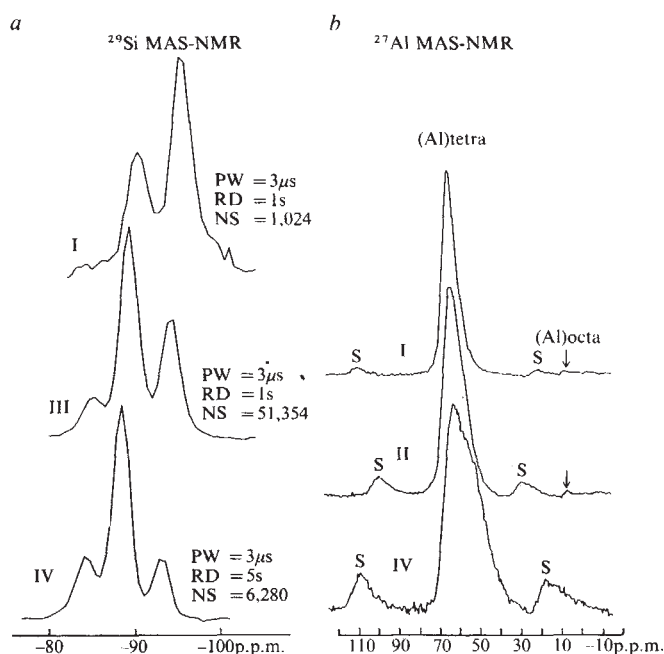


Fig. 1 a, ²⁹Si and b, ²⁷Al MAS-NMR spectra at 79.5 and 104.2 MHz, respectively, for synthetic 2:1 clays. Spinning frequencies were 4–5 kHz. Cross-polarization was not used. The pulse width (PW), relaxation delay (RD) and number of scans (NS) are provided for each ²⁹Si spectrum; the PW for a 90° flip angle was 8 μs. For the ²⁷Al spectra PW = 3 μs, RD = 40 ms, NS = 1,024. The ²⁹Si spectra were calibrated using pyrophyllite with a chemical shift of -95 p.p.m. upfield from TMS¹⁵. The ²⁷Al chemical shifts are relative to Al(H₂O)₆³⁺. The lines labelled S are spinning sidebands.

in samples (I) and (II). However, the intensities of the octahedral Al resonances are much lower than expected from the unit cell compositions given in Table 1. Experimental pulse widths and relaxation delay times were varied over the range 3–300 μs and 0.004–2.05 s, respectively, in an unproductive attempt to improve the strength of the octahedral signal.

An analysis of the ²⁹Si MAS-NMR data, presented below, shows that our clays contain tetrahedral Al in which the near-neighbour tetrahedral sites are occupied by Si, that is, all of the tetrahedral Al is in (0 Al) environments. It is possible, therefore, that the multiple tetrahedral ²⁷Al resonances arise from differences in the occupancy of second near-neighbour tetrahedral sites. Although the octahedral Al in pyrophyllite and other 2:1 layered silicates is readily observed by ²⁷Al MAS-NMR at high magnetic field strengths, the unusually weak resonance in our samples, which are tetrahedrally charged trioctahedral structures, may be associated with a large and non-uniform electric field gradient at the octahedral sites.

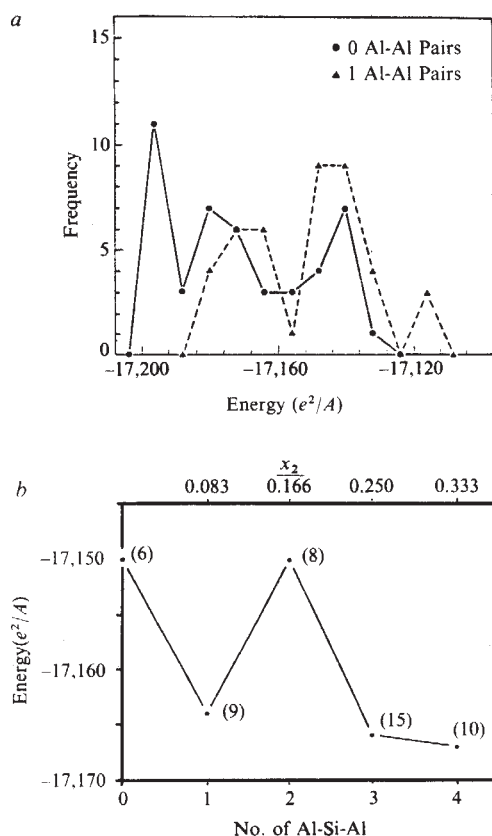


Fig. 2 a, The potential energy distribution obtained for 89 randomly generated mica-like structures ($x = 0.250$), grouped according to the presence of 0 (●) and 1 (▲) Al-Al linkages per unit cell [$e^2/A = 14.4$ eV]. b, The dependence of the average potential energies of those mica-like structures with $x_1 = 0$ on the number of Al-Si-Al linkages per unit cell. The numbers in parenthesis indicate the number of structures contributing to the average energies.

The tetrahedral site distribution in 2:1 layered silicates can be obtained from the ^{29}Si NMR intensities by an analysis similar to that made for zeolites¹¹. The fraction of tetrahedral Al-Al linkages x_1 , is given by $x_1 = 3x/2 - (1-x)M_1/2$, where x is the fraction of sites occupied by Al, $M_1 = (I_1 + 2I_2 + 3I_3)$, and the I_i are the normalized areas of the NMR lines due to ^{29}Si with $i = 0, 1, 2$, and 3 Al neighbours. If Loewenstein's rule holds, $x_1 = 0$, then the fraction of tetrahedral sites occupied by Al can be obtained from the NMR spectra through the expression $x = M_1/(M_1 + 3)$.

The fraction of Si sites involved in Al-Si-Al linkages, x_2 , is given by the expression $x_2 = (1-x)M_2$, where $M_2 = (I_2 + 3I_3)$. For a random distribution of Si and Al in tetrahedral sites, subject to Loewenstein's rule, the probabilities of finding 0, 1, 2 or 3 Al tetrahedra adjacent to a Si can be calculated. If $(\text{Si}/\text{Al})_{\text{tet}} = 1/r$, the probabilities P_i , $i = 0, 1, 2$, or 3 Al, are given by the binomial distribution $P_0 = (1-r)^3$, $P_1 = 3r(1-r)^2$, $P_2 = 3r^2(1-r)$ and $P_3 = r^3$. The ^{29}Si NMR spectrum for this random distribution model consists of four lines with normalized intensities given by the P_i above.

The values of the experimental ^{29}Si -NMR line intensities and site distribution parameters defined above are given in Table 2. The values of x_1 are small and fluctuate about zero, showing that within experimental uncertainty, Loewenstein's rule is obeyed for this series of synthetic clays over the composition range $x = 0.115$ – 0.267 . The agreement between the values of x determined by chemical analysis and by NMR analysis also is compatible with Loewenstein's rule. The experimental values of x_2 are always smaller than those predicted by the random

Table 2 ^{29}Si MAS-NMR line intensities and tetrahedral site distribution parameters

Sample	I_0	I_1	I_2	x	x_1	x_2
(I)	0.61	0.39	—	0.115 (0.11)	0.00	0.00 (0.04)
(II)	0.52	0.44	0.04	0.170 (0.16)	0.04	0.04 (0.11)
(III)	0.35	0.55	0.10	0.186 (0.20)	−0.03	0.08 (0.12)
(IV)	0.19	0.57	0.24	0.267 (0.26)	0.02	0.18 (0.30)

The normalized integral line intensities (I_0 , I_1 , I_2) were obtained by fitting the observed spectra to a sum of three independent gaussian lines using a least-squares method. Values of x in parentheses were determined from the NMR line intensities, assuming Loewenstein's rule. Values of x_2 in parentheses were calculated from the random distribution model.

distribution model which indicates there is some degree of short-range order in tetrahedral Al and Si sites in these structures.

To understand the factors influencing the values of x_1 and x_2 , particularly in the case of the mica-like sample (IV), we have carried out electrostatic potential energy calculations^{12,13} on a mica structure ($x = 0.250$) with different tetrahedral Al and Si arrangements. The unit cell selected contained 16 tetrahedral sites filled by 12 Si and 4 Al. There are 1,820 different ways to fill the 16 sites. Of these, 100 were randomly selected and a crystal structure for each was derived by the DLS method¹⁴. Because the structural OH dipole is sensitive to cation distribution, OH was replaced by F in the model structures.

The distribution of energies for 89 of the 100 structures is shown in Fig. 2a where the structures are grouped according to the presence of 0 or 1 Al-Al linkages per unit cell. The remaining 11 structures, which include 2 Al-Al linkages per unit cell, have energies above $-17,090$ e^2/A . There is a marked tendency for the potential energy to favour structures in which no such linkages are present (that is $x_1 = 0$). This suggests Al ions will occupy adjacent tetrahedra only rarely, in accord with Loewenstein's rule and the experimental ^{29}Si MAS-NMR results of the present work.

Figure 2b illustrates the dependence of the average potential energy of those structures for which $x_1 = 0$ (48 of the 89 structures) on the number of Al-Si-Al linkages per unit cell. These results show that the structures with one and four Al-Si-Al linkages (that is $x_2 = 0.083$ and 0.333) are more stable than structures containing zero and two Al-Si-Al linkages ($x_2 = 0$ and 0.166). Because of the small number of tetrahedra considered in the unit cell, the sampling of x_2 values is rather coarse. More extensive calculations with more sites per unit cell will be needed to predict accurately the most stable x_2 value.

We conclude that ^{29}Si MAS-NMR measurements on four synthetic 2:1 clays have shown that the Al tetrahedral occupancy follows Loewenstein's rule. Furthermore, by determining x_2 , the number of Al-Si-Al linkages per Si, we have shown that the Al occupancy is not random, there must be short-range ordering. The calculations for a mica with $(\text{Si}/\text{Al})_{\text{tet}} = 3$ are consistent with this result as they show a stable configuration with an x_2 value considerably lower than that given by the random distribution model. We believe this is the first reliable demonstration of short-range ordering in 2:1 phyllosilicates.

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Nets with channels of unlimited diameter

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Three-dimensional nets and polyhedra^{1,2} present an unlimited mathematical challenge, and systematic enumeration of the infinite series of four-connected nets is just developing^{3–10} using selected algorithms. Several framework structures were solved and classified by invention of new nets (see refs 11–13). The four-connected nets of low density are of practical interest because their nodes may correspond with the tetrahedrally-coordinated atoms of aluminosilicate zeolites¹⁴, some silica polymorphs¹⁵, aluminophosphates^{16,17} and other molecular sieves. The nets can be classified by circuit symbols^{1–7} and coordination sequences^{18,19}. Each oxygen atom of a zeolite and other tetrahedral frameworks lies near the midpoint of a branch connecting two tetrahedral nodes, and the empty volume between the close-packed oxygen atoms is important for molecular sorption. The largest ring in a natural zeolite or synthetic molecular sieve involves 12 tetrahedra^{11,14}, but larger rings and sphere packings of lower density are known^{12,20–24}. We report here two new infinite series of four-connected three-dimensional nets which contain channels of unlimited diameter.

The four-connected three-dimensional net number 39 was obtained⁴ from a stack of 4.8² three-connected two-dimensional nets by addition of alternating upward and downward branches. Figure 1a uses absence or presence of a dot at each three-connected node of the horizontal two-dimensional net to represent the colouring produced by the change of vertical direction of the fourth branch. Each single four-ring (or square) can be extended to an unlimited band of $(n+1)$ four-rings for which the simpler examples with $n=1$ and 2 are shown in Fig. 1b and c. Correspondingly, the channel diameter in the three-dimensional net expands steadily as the limiting ring increases from eight nodes (a) to 12 (b), to (16) (c) and in general to

$4(n+2)$. Relative rotation of adjacent bands of four-rings would allow an elliptical shape for the channels.

Similar alternation of vertical branches joining a stack of 4.6.12 three-connected two-dimensional nets⁴ gave four-connected three-dimensional net number 81 (Fig. 2a) which is represented by the fifth member¹⁷ of a series of aluminophosphate molecular sieves. Conversion of each four-ring into a band of four-rings produces a second infinite series for which the simpler examples with $n=1$ and 2 are shown in Fig. 2b and c. As n increases, the limiting ring of the channel expands from 12 nodes for one four-ring to 18 ($n=1$), to 24 ($n=2$), and to $6(n+2)$ in general.

Strict alternation of the vertical branches is maintained in both series, and the highest symmetry is assumed for each value of n . Sub-series with lower symmetry, and with incongruent channels, can be developed by variation of n in different horizontal directions. The strict alternation of vertical branches converts each horizontal branch into a crankshaft chain³. Adjacent crankshaft chains around the surface of each near-cylindrical channel generate edge-shared hexagons (or six-rings) which form a 6³ cylindrical net (stereoplot in Fig. 4 of ref. 17; CAN channel in Fig. 3 of ref. 25). Further infinite series with channels of unlimited diameter can be obtained by changing the sequence of vertical branches⁴ and the type of vertical chain⁵; furthermore, complex cages are obtained by rotation of some branches as in net 83 of ref. 4. Another suite of infinite series can be obtained from the two-dimensional net (4.6.8)(4.8.12) found in mazzite and zeolite L. Full details will be published elsewhere.

Table 1 lists the geometrical and numerical properties of the nets in Figs 1 and 2, based on the most regular geometry and an internodal distance near 0.31 nm. It is assumed that each crankshaft chain is flexed so that the repeat distance along the chain is 0.85 nm as in aluminophosphate no. 5 (ref. 17), and that the horizontal branches of the two-dimensional net are tilted. The free diameter of each near-cylindrical channel assumes that oxygen atoms (radius 0.14 nm) are placed in positions similar to those of aluminophosphate no. 5. In a real framework, for example, of Si₂O₄ composition, the geometrical details would depend on the chemical bonding, and there is some tolerance in the distances given in Table 1. For a framework of AlPO₄ composition with alternation of Al and P, the topochemical symmetry is lower than the topological symmetry, as in the fifth member of the AlPO₄ series¹⁷.

Direct comparison with ideal sphere packings is difficult because the latter have constant distances between adjacent spheres in contrast to the considerable variation of O–O distances in the silica polymorphs and the aluminosilicate and aluminophosphate molecular sieves. Nevertheless, it is instructive to compare the density of the present series of open nets with the least dense sphere-packings in refs 20–24. First, the density of four-connected nodes decreases systematically towards zero as n increases towards infinity for each of the series based on nets 39 and 81. For T–T = 0.31 nm, the sequence of node density per nm³ as n increases from 0 in nets 39(n) is

Fig. 1 Two-dimensional projections of the first three members of an infinite series of three-dimensional nets based on strict alternation of the vertical direction (presence or absence of dot) of a fourth branch added to each node of the three-connected 4.8² net. Each net is shown with the most regular geometry and highest topological symmetry. The unit cell is shown by corner marks.

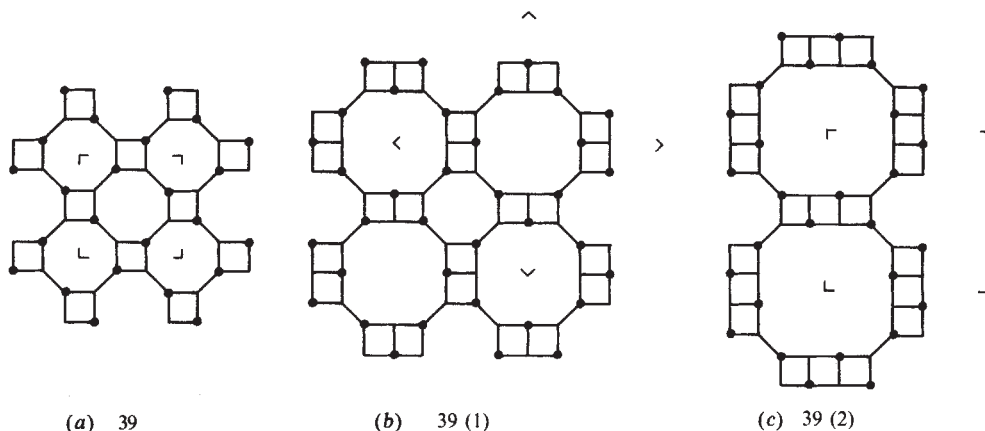


Table 1 Properties of three-dimensional nets in ideal expanded shape

Arbitrary no.	Z_t	Circuit symbol	Space group	Z_c	a (nm)	c (nm)	Free diameter (nm)
39	8	4.6^5	I4/mcm	16	1.0 ₀	0.8 ₅	0.3
39 (1)	24	$(4^2 6^4)_1(4.6^5)_2$	I4/mcm	48	1.8 ₇	0.8 ₅	0.6
39 (2)	32	$(4^2 6^4)_1(4.6^5)_1$	P4/mcc	32	1.6 ₀	0.8 ₅	0.9
39 (3)	40	$(4^2 6^4)_3(4.6^5)_2$	I4/mcm	80	2.7 ₁	0.8 ₅	1.2
39 (n)	$8(2+n)$	$(4^2 6^4)_n(4.6^5)_2$	*	*	*	0.8 ₅	$0.3(n+1)$
81	24	4.6^5	P6 ₃ /mcc	24	1.37 [†]	0.85 [†]	0.72 [†]
81 (1)	36	$(4^2 6^4)_1(4.6^5)_2$	P6 ₃ /mcm	36	1.8 ₇	0.8 ₅	1.2
81 (2)	48	$(4^2 6^4)_1(4.6^5)_1$	P6 ₃ /mcc	48	2.3 ₇	0.8 ₅	1.7
81 (n)	$12(2+n)$	$(4^2 6^4)_{n-2}(4.6^5)_2$	‡	$12(2+n)$	$(1.37+0.5n)$	0.8 ₅	$(0.72+0.5n)$

For ease of reference, each sequence of nets is given the arbitrary number of the prototype from ref. 4 plus a bracketed number for the added four-rings. All estimated distances are analogous to those observed for aluminophosphate no. 5, and a range of several percentage can be obtained by varying interatomic angles. Z_t and Z_c are the number of four-connected nodes in the unique unit and the unit cell, respectively.

* n even: P4/mcc, $8(2+n)$, $1.0+0.3n$; n odd: I4/mcm, $16(2+n)$, $1.4_{75}+0.4_{25}n$.

† Observed values for aluminophosphate no. 5. All other distances depend on assumptions about bond angles.

‡ n even: P6₃/mcc; n odd: P6₃/mcm.

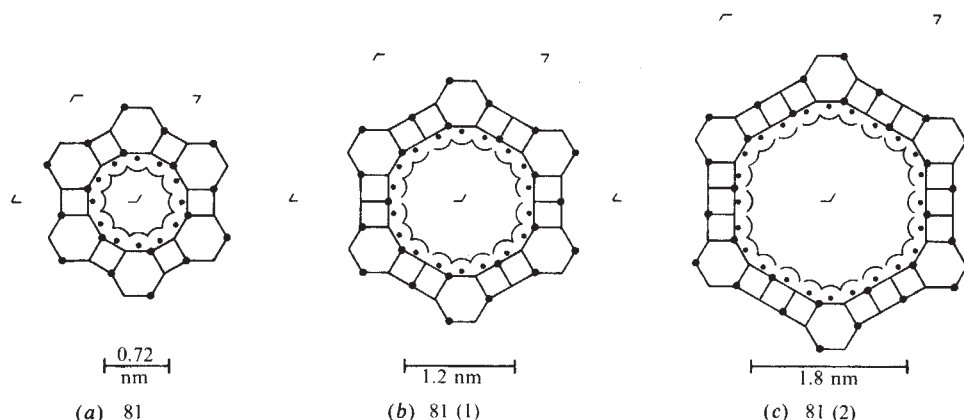


Fig. 2 Two-dimensional projections of the first three members of an infinite series of three-dimensional nets based on strict alternation of the vertical direction (presence or absence of large dot) of a fourth branch added to each node of the three-connected 4.6.12 net. The unit cell is shown by corner marks. Small dots show approximate positions of oxygen atoms expected for occupancy of the tetrahedral nodes by Si or by alternating Al and P, and the arcs represent an atomic radius of 0.14 nm. The approximate free diameter for a molecular sieve is shown below each net.

18.8, 16.1, 14.7, 13.0, 11.7, 10.5, 9.6... Similarly for nets 81(n), the sequence is 17.4, 14.0, 11.6... $16.3(2+n)/(1.37+0.5n)^2$. There is also no lower limit of density for the nets²⁴ constructed by the Heesch-Laves²⁰ procedure in which a sphere is replaced by a ring of three spheres in a sphere packing.

From the viewpoint of molecular-sieve technology, the new series of nets offer an opportunity of unlimited channel diameter if synthesis can be achieved, and the products are robust. Various large ions have proved useful as templates²⁶ (for example, tetrapropylammonium in the synthesis of fluoride-silicalite²⁷), and further exploration of large organic species, including chains and helices, should be considered. Heteropoly and isopoly complexes of the transition metals of groups 5 and 6 (ref. 28) might also be considered if suitable conditions (including pH) can be found for crystallization and removal of the occluded species. The existence of large channels (1.5 nm free diameter) in the mineral cacoxenite²⁹ should be encouraging, even though iron (III) oxygen octahedra do not provide a suitable model for tetrahedra. The 81(n) series of nets is particularly rigid, and might prove useful in engineering design. Even if the new nets have no practical value, they extend the range of mathematical theory, and provide new opportunities for artistic development.

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Quasi-liquid crystals

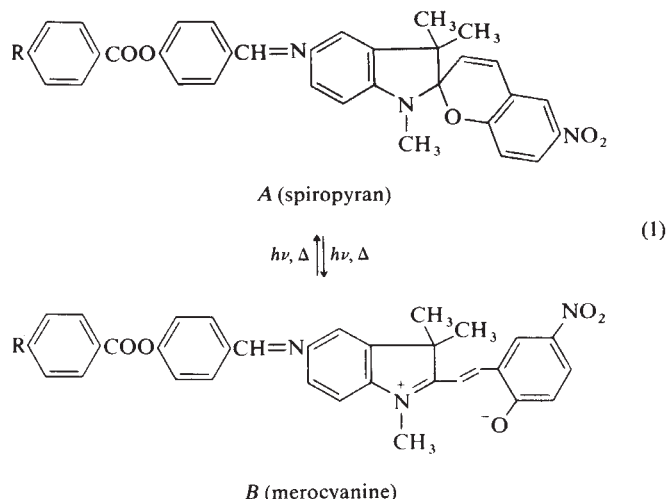
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Photo- and thermochromic conversion of spiropyrans into merocyanines¹ is used to 'engineer' new types of molecular organizes²⁻⁶, based on the capability of the merocyanine dyes to give giant molecular stacks⁷. One type of spiropyran-merocyanine organize, the so-called quasi-crystal, is formed when the photochromic reaction occurs in an electrostatic field. The quasi-crystals are composed of assemblies of dipolar molecular stacks covered by amorphous materials and aligned along the field^{2,3}.

Quasi-crystals exhibit optical nonlinearity⁴. Other types of organizes occur in macromolecules bearing spiropyran side groups^{5,6}. Both inter- and intramolecular stacking of merocyanine side groups have been disclosed. Here we describe a new spiropyran-merocyanine organize obtained in spiropyran containing mesogenic groups. These materials exhibit some features of liquid crystals (birefringence, orientation in an electric field), but have structures that are completely different from conventional liquid crystals. We propose that organic compounds with this novel form of organization be called quasi-liquid crystals (QLCs).

Spiropyrans of the general formula A ($R = \text{CH}_3\text{O}-$, $n-\text{C}_6\text{H}_{13}\text{O}-$, $\text{CN}-$, see below) were synthesized by a process that will be described elsewhere. The structures and purities (> 99%) of the spiropyrans were established by chemical analysis, NMR and mass-spectroscopy. The colour changes of these materials are associated with the following reversible reaction¹:



Crystals of these spiropyran, obtained by slow crystallization from a suitable solvent, have sharp melting points (Table 1) and give isotropic melts.

The compounds can also be obtained as thin amorphous films by fast evaporation of the solvent from their solutions on a glass surface. In this way we prepared very viscous (grease-like) transparent films of $\sim 0.5 \mu\text{m}$ thickness. They are metastable and remain in the amorphous state for between several hours and several days, depending on their thickness, temperature of preparation and the substituent R. The amorphous films obtained from mixtures of spiropyran proved to be much more stable. We ascribe the formation of the amorphous films to the presence, at low concentrations, of merocyanine molecules. This merocyanine apparently acts as an impurity, retarding crystallization of the films.

Heating the amorphous films induces a change to an anisotropic structure, observable in the polarization microscope through its strong birefringent texture (Fig. 1a). The appearance of this texture coincides with a substantial change in the film colour, from pale yellow to bright green or greenish-blue. The change in colour is associated with a shift of the thermal equilibrium (1) to the right, with increasing temperatures. At a still higher temperature this texture disappears: above this clearing point total extinction of light is observed through cross polarizers. The temperature range in which the textured structure exists is much lower than the melting point (m.p.) of the crystals (Table 1). For example, for the spiropyran with $R = \text{CH}_3\text{O}-$ the m.p. is $198-203^\circ\text{C}$, while the temperature range for the birefringent texture is $50-130^\circ\text{C}$. The material above 130°C is an isotropic liquid; below the melting point of the crystal it is metastable. The texture disappears above 130°C , and reappears on cooling below 130°C . However, this process is not completely reversible due to the competitive crystallization process, which is slow at low temperatures but faster at higher temperatures. The beginning of this crystallization process is shown in Fig. 1b. The clusters of non-birefringent dark spots are microcrystals.

Films cooled to room temperature retain their anisotropic structure. It looks as if the mesophase is 'hidden' beneath the crystal phase and appears only when we destroy the crystal lattice by dissolving the crystalline spiropyran. However, this is not the case, because the isotropic material obtained by heating spiropyran crystals above the melting point gives, on cooling, isotropic glass, which does not exhibit birefringence on being reheated from room temperature, that is the system does not exhibit properties of monotropic mesophases.

For orientation in an electric field a thin amorphous film was cast onto a glass slide with interdigital electrodes. The QLC were orientated in a constant electric field of $> 0.5 \text{ kV mm}^{-1}$. For example, films of spiropyran with $R = \text{CH}_3\text{O}-$ give at 90°C and $E = 1.5 \text{ kV mm}^{-1}$ nearly homogeneous birefringence (Fig. 2). The higher the field and the temperature (below the clearing point) the better and more uniform was the orientation determined from the linear dichroism. Very thin films ($< 1 \mu\text{m}$) are oriented much more slowly and less completely, probably due to an anchoring effect. Orientation in the field was also observed on the hydrophobic surface obtained by treating the glass with a solution of octadecyltrichlorosilane in bicyclohexyl.

The most remarkable effect of the electrostatic field is the stabilization of the quasi-liquid crystalline state which accompanies the orientation of the films. Spontaneous crystallization no longer occurs. In the supercooled conditions at room temperature the orientation and glass-like state were preserved without change for at least 3 months after the field was switched off. Both electron microscope results (to be published) and optical microscope observations showed the absence of crystals. However, at temperatures above the clearing point the electric field does not cause orientation but on recooling in the field the orientation is restored.

Stabilization of the oriented supercooled films allowed us to measure the absorption spectra of the QLC with polarized light (Fig. 3). The extinction coefficients of the spiropyran (ϵ_{sp}) were measured in solution in benzene (Fig. 3). They are, at 370 nm , 2.23×10^4 and 1.75×10^4 for the compounds with $R = \text{CH}_3\text{O}-$ and $R = \text{CN}-$, respectively. The extinction coefficients at the visible absorption maxima (ϵ_{mer}) of the merocyanines obtained from different spiropyran lie in the rather narrow range $(3-5) \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$ (refs 1, 2). Assuming these coefficients to be valid for the films we can estimate the ratio of the concentrations (C) of merocyanine and spiropyran in these films from the absorption spectra: $C_{\text{mer}}/C_{\text{sp}} = (\epsilon_{\text{sp}}/\epsilon_{\text{mer}}) (D_{\text{mer}}/D_{\text{sp}})$, where D_{mer} and D_{sp} are the absorptions of merocyanine and spiropyran, respectively. It is estimated that the extent of conversion of spiropyran into merocyanine on heating for different films is only 3–10%. A further, most surprising, fact is that the absorption spectra of the films are dichroic only in the region of the merocyanine absorption band ($\lambda_{\text{max}} \sim 600 \text{ nm}$), while in the region of spiropyran absorption ($\lambda_{\text{max}} \sim 370 \text{ nm}$) the degree of linear dichroism is vanishingly small. This means that only the merocyanine molecules are oriented in the electric field,

Table 1 Characteristics of spiropyran

Compound and mixture	Melting point* (°C)	Temperature ranges of phases (°C)	
		Amorphous → QLC → Isotropic	
I	198–203	50	130
II	165–168	45	110
III	210–214	80	170
I:II = 3:1	—	50	135
I:III = 1:1	—	60	140

* Not corrected.

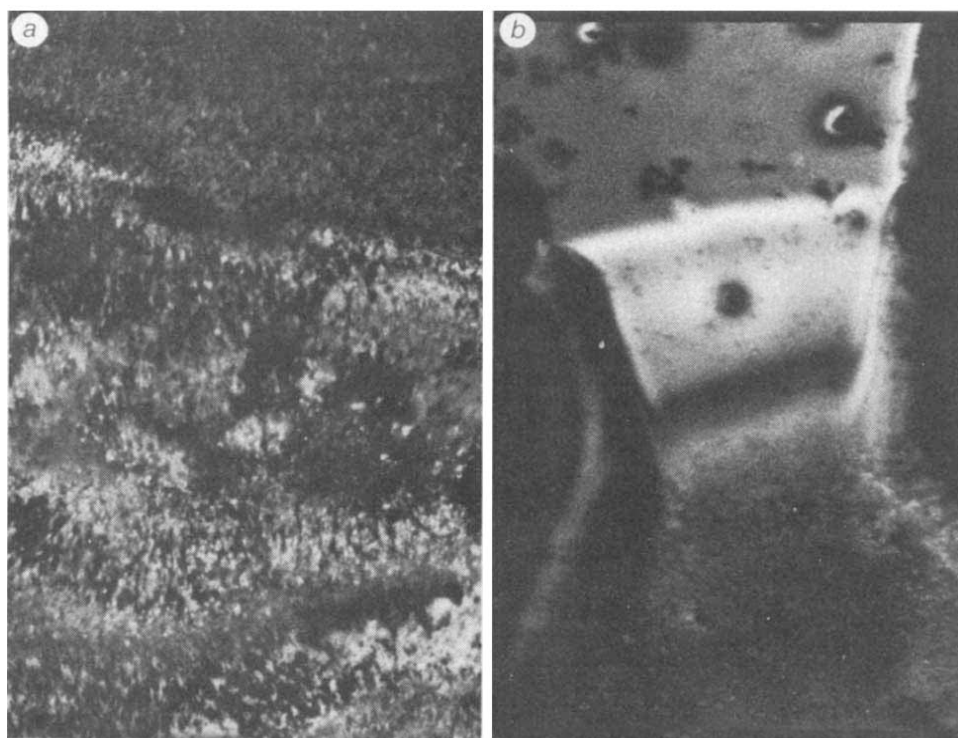


Fig. 1 Optical texture of unaligned QLC films viewed through crossed polarizers. *a*, Mixture of spiropyrans with $R = \text{CH}_3\text{O}-$ and $\text{CN}-$, in the ratio 1:2, at 115°C . *b*, Spiropyran with $R = \text{CH}_3\text{O}-$, at 90°C ; clusters of non-birefringent microcrystals are distinctly seen on the yellow and bluish-grey sections as shown on the front cover of this issue.

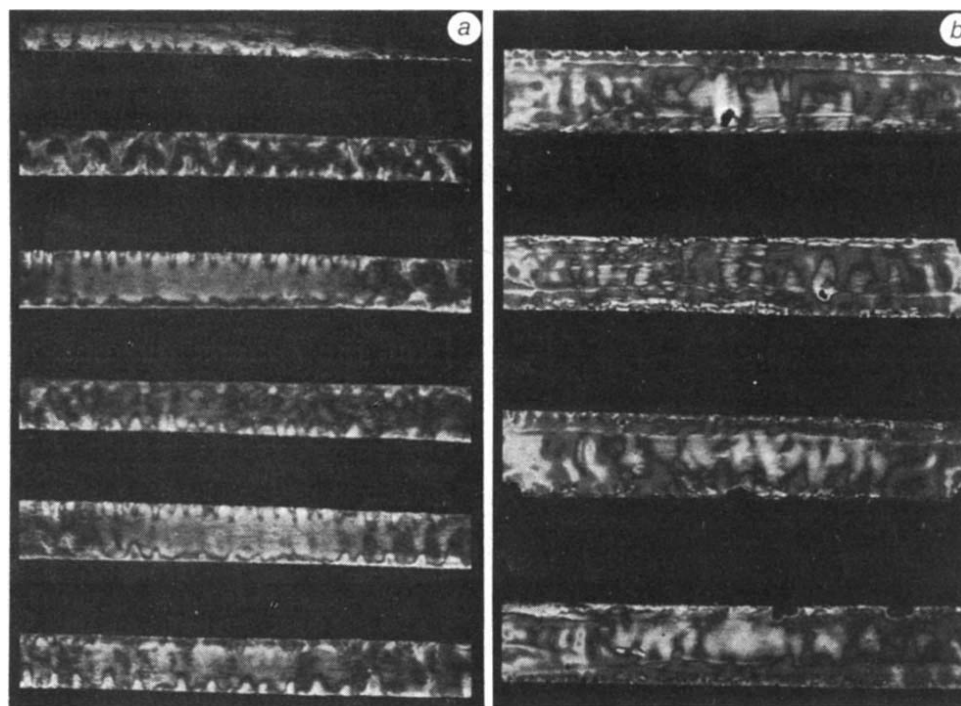


Fig. 2 Optical texture of QLC films aligned in an electrostatic field ($E = 1.5 \text{ kV mm}^{-1}$, at 90°C , distance between electrodes = 1 mm), viewed through crossed polarizers. The dark parallel stripes on the picture are areas of the interdigital electrodes. *a*, Mixture of spiropyrans with $R = \text{CH}_3\text{O}-$ and $\text{CN}-$, in the ratio 1:2. *b*, Spiropyran with $R = \text{CH}_3\text{O}-$.

while the bulk of the material, which consists of spiropyran molecules, is unoriented.

The order parameter of the merocyanines is given by $S = (D_{\parallel} - D_{\perp}) / (2D_{\perp} + D_{\parallel})$, where $D_{\parallel}$ and $D_{\perp}$ are, respectively, the absorptions parallel and perpendicular to the electric field. This parameter depends on the field strength, the temperature and the film thickness. For example, a 1:2 mixture of spiropyrans

with $R = \text{CH}_3\text{O}-$ and $\text{CN}-$ gives $S \approx 0.2$ at 90°C and $E = 1.5 \text{ kV mm}^{-1}$. The direction of the long molecular axis, coincident with the direction of maximum absorption of polarized light, is parallel to the field for all examined compounds and mixtures.

Orientation of the films in a low-frequency alternating field ($\sim 50 \text{ Hz}$) was also observed, but in this case the oriented QLC were not stable and crystallization occurred very rapidly.

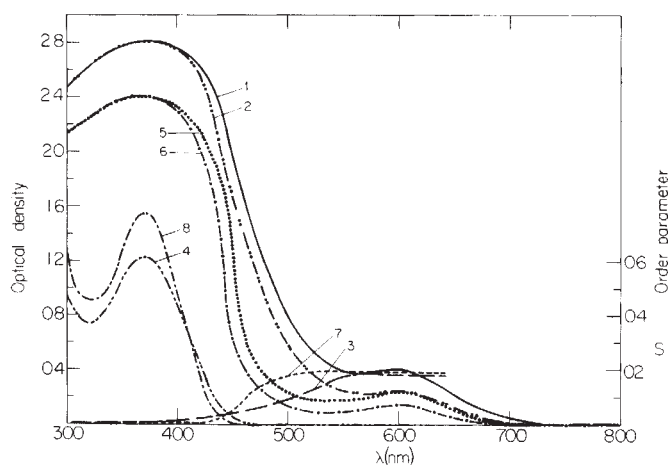


Fig. 3 1–4, Spiropyran with $R = \text{CN}-$: 1,2-absorption spectra of oriented QLC films, light polarized respectively parallel and perpendicular to the electric field; 3, order parameter, S , as a function of λ ; 4, absorption spectrum in benzene solution. 5–8, As 1–4, but for spiropyran with $R = \text{CH}_3\text{O}-$.

Simple considerations, similar to those given in refs 2 and 3, lead us to conclude that orientation of separate merocyanine molecules is inconceivable in an electric field as weak as $1-1.5 \text{ kV mm}^{-1}$. Only assemblies of interacting molecules, having large net dipole moments, can be oriented in such a field. It is also inconceivable that the weak interactions, associated with the anisotropy of the polarizabilities of the mesogenic groups, and responsible for the occurrence of the liquid crystalline state of material, would keep together the merocyanine molecules in such assemblies. Indeed, the concentration of merocyanines in the isotropic spiropyran bulk is low (3–10%) and the existence of liquid crystalline domains of merocyanines in a predominantly isotropic phase is thermodynamically highly improbable.

Apparently the merocyanine molecules with the mesogenic groups somehow impose mesophase features on the bulk material: very intensive birefringence in the liquid phase, which indicates ordering of the material and characteristic temperature behaviour. These properties are not inherent in the spiropyran-merocyanines organizes that we studied previously. It is improbable that in the QLC the merocyanines or their complexes are organized in molecular stacks similar to those in quasicrystals or in 'zipper' polymer crystals because the viscosity of the material is extremely high and stack formation by diffusion would be extremely slow. Rather, the interaction of the dipolar merocyanine molecules with the surrounding spiropyran results in formation of small domains similar to solvating shells or to the asymmetric micelles in lyotropic systems⁸. If these domains interact with one other they may provide the material with QLC properties. On raising the temperature above the clearing point the ordering of the domains is presumably destroyed. The fact that QLCs appear only in the films cast from solution and never from spiropyran melt may indicate some kind of latent preorganization or complexation developing on evaporation of solvent.

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Sapropelic deposits in a sediment from the Guinea Basin, South Atlantic

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Until recently it has been generally thought that a stagnant water column is a prerequisite to the formation of organic-rich deposits in deep-water marine sediments, a belief best exemplified by studies on the eastern Mediterranean sapropels. Many workers have assumed that, following the hydrological isolation of the deep waters, stagnation of the eastern Mediterranean basins and the concomitant development of anoxic conditions occurred before the deposition of the sapropelic layers^{1–10}. Opposing arguments suggesting a role for increased marine productivity have, however, been put forward, both specifically with respect to the eastern Mediterranean sapropels^{11,12} and more generally regarding deep-water organic-rich deposits^{13–15}. We report here the discovery of sapropelic layers ($>0.5\%$ organic carbon)¹⁶ in a recent sediment core from a deep-water site in the Guinea Basin. It is thought most unlikely that stagnant conditions could prevail in the water column at this site, and preliminary analyses of the sediments suggest that the organic-rich deposits have been a direct result of increased productivity in the overlying water column, the most recent occurring about 18,000 yr ago and coinciding with the last glacial maximum.

The core (10529) was collected using a Kastenlot corer¹⁷ equipped with a 4-m long $15 \times 15 \text{ cm}$ section barrel during RRS *Discovery* 128 cruise to the South Atlantic. The core site was on the flat abyssal plain of the Guinea Basin ($4^{\circ}56.8' \text{ S } 0^{\circ}27.7' \text{ E}$, water depth 4,735 m). The sediment core consisted of alternating marls and oozes. The oozes were grey to black in colour and distinct black-grey bands were found in each of the ooze deposits (Fig. 1).

A microscopic analysis indicated large numbers of Radiolaria and large amounts of siliceous (mainly diatom) debris in the oozes. There appeared to have been a considerable variation in the degree of carbonate dissolution throughout the core but no obvious relationship between dissolution and either the marl or ooze deposits, although particularly strong dissolution was seen at the base of the lowest ooze deposit (350 cm).

The results of a stratigraphical analysis based on coccolith distributions is shown in Fig. 2. Relative percentages of *Emiliania huxleyi* and *Gephyrocapsa* spp. have been used to determine ages. The reversal in dominance between *G. oceanica* and *E. huxleyi* is known to occur, in tropical areas, at about 85,000 yr whilst the first occurrence of *E. huxleyi* is dated at 270,000 yr (ref. 18). The peak abundance of *G. aperta* between these two dates has been recorded by Breheret¹⁹ and Weaver²⁰ and it occurs in oxygen isotope stage 7. No evidence of any major sediment reworking, such as by turbidites, was found. Any such sediments would contain mixed young and old coccolith assemblages which were not present in the samples analysed in this work. The fact that the distributions closely match those found in other studies^{18,20} suggests that the core is stratigraphically intact with an average sedimentation rate of 1.4 cm kyr^{-1} .

Organic carbon, carbonate and X-ray diffraction analyses²¹ show the oozes to have considerably raised levels of organic carbon (Fig. 1) and opaline silica (Fig. 3a), but much reduced amounts of calcium carbonate. The calcium carbonate values were confirmed by X-ray emission analysis¹¹ for CaO (Fig. 1). The three major ooze deposit coincide with peaks in the organic carbon values with a double peak in the most recent ooze. The low carbonate values in the oozes could be caused either by dissolution or dilution of the carbonate fraction. Visually there appeared to be no consistent relationship between dissolution of carbonate tests and the ooze deposits. Furthermore plotting the organic carbon and opal values on a carbonate-free basis

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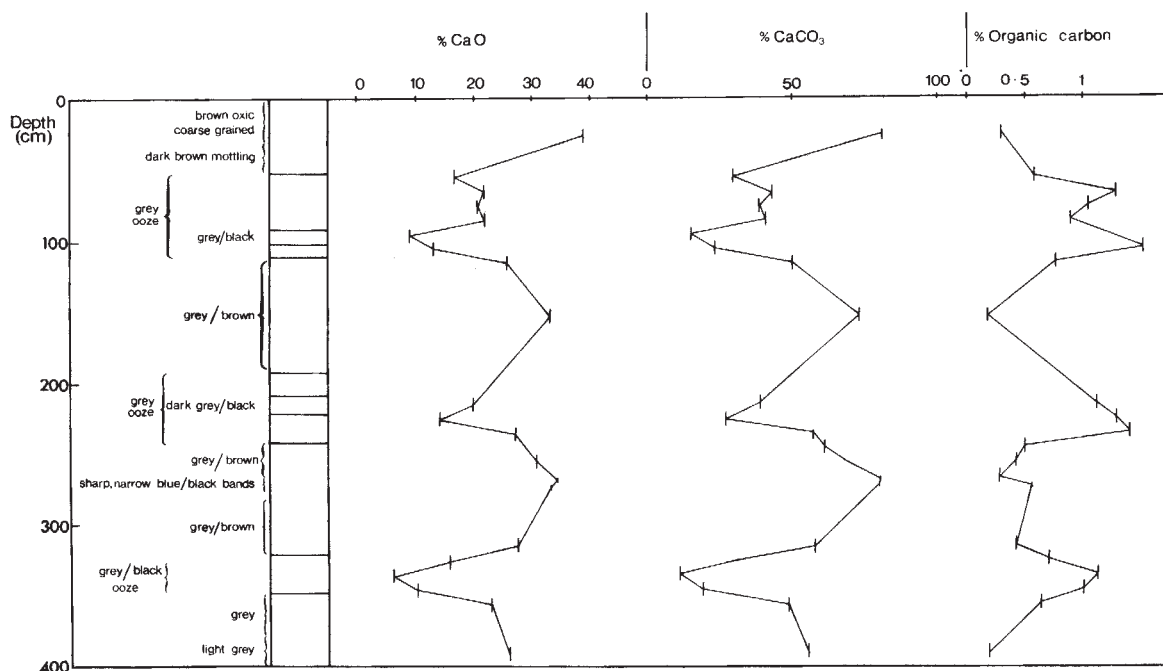


Fig. 1 Core description and content of organic carbon carbonate (% dry weight of sediment).

(Fig. 3b) indicates that the variation in these components are independent of variations in the carbonate content. The available evidence suggests that the increased levels in organic carbon and opaline silica reflect periods of increased inputs of biogenic silica to the sediments diluting the normal carbonate contribution.

Quartz levels in the core are generally low (<1%) but X-ray emission spectrometric analyses^{11,12} indicate that there have been increased contributions of fine-grained aluminosilicate minerals to the sediment during ooze deposition (Fig. 3a). The origins of these inputs are at present unknown, but again there is no evidence of major post-depositional sediment transport (such as turbidites) in the core.

Some 10-cm horizons from the upper part of the core were taken for ^{14}C dating and $\delta^{13}\text{C}$ measurements (Table 1). Age data based on carbonate ^{14}C give no indications of any major contribution by turbidites in the upper 90 cm of the core, in agreement with the stratigraphical analysis (below 90 cm the age of the core is beyond the ^{14}C dating method). The ^{14}C carbonate ages give accumulation rates of between 0.8 and 3.4 cm kyr⁻¹.

Sufficient organic carbon was present in the 60–70 cm and 70–80 cm depth horizons for the determination of the component ^{14}C organic ages (Table 1). The most recent organic carbon enrichment (60–70 cm) coincides with the last (Stage 2) glacial maximum, taken here to be 14,000–19,000 yr BP. The determined organic carbon ages are considerably younger than the corresponding ^{14}C carbonate ages. This effect, though not normally to this extent, has been frequently noted²² and has been attributed to the reworking of old carbonate particles into contemporaneous sediment¹¹. In the present work, the coarseness of the sample horizon is thought to be another factor. The determined ages are an average of a 10-cm band, yet within these horizons a range of sedimentation could have occurred with the relatively steady carbonate sedimentation being periodically supplemented by shorter episodes of organic sedimentation. Turbidites are not thought to be an explanation for the discrepancy between the ^{14}C carbonate ages and ^{14}C organic ages; any turbidite action would have caused anomalies in both sets of age determinations.

The $\delta^{13}\text{C}$ measurements suggest that the organic material in the 60–70 cm and 70–80 cm horizons is derived overwhelmingly from marine planktonic sources^{11,20}. This is further evidence against turbidite action; the continental shelves and slopes around the Niger delta are the most likely sources for turbidites

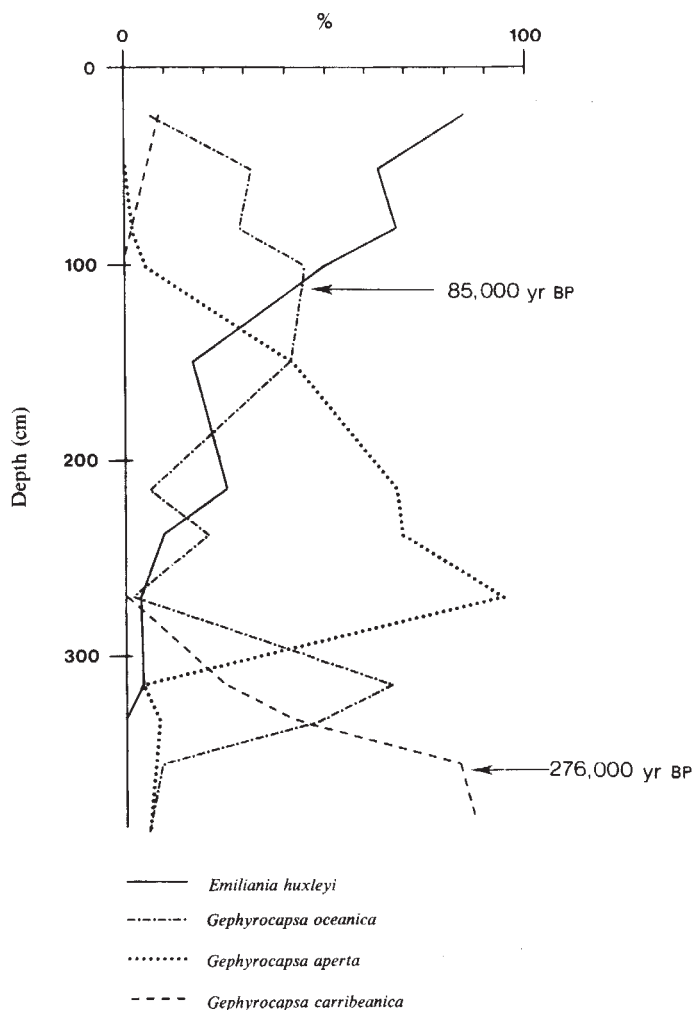


Fig. 2 Coccolith stratigraphy. The distributions suggest continuous deposition to beyond 276,000 yr. The two dates in the right hand column are derived from ref. 17.

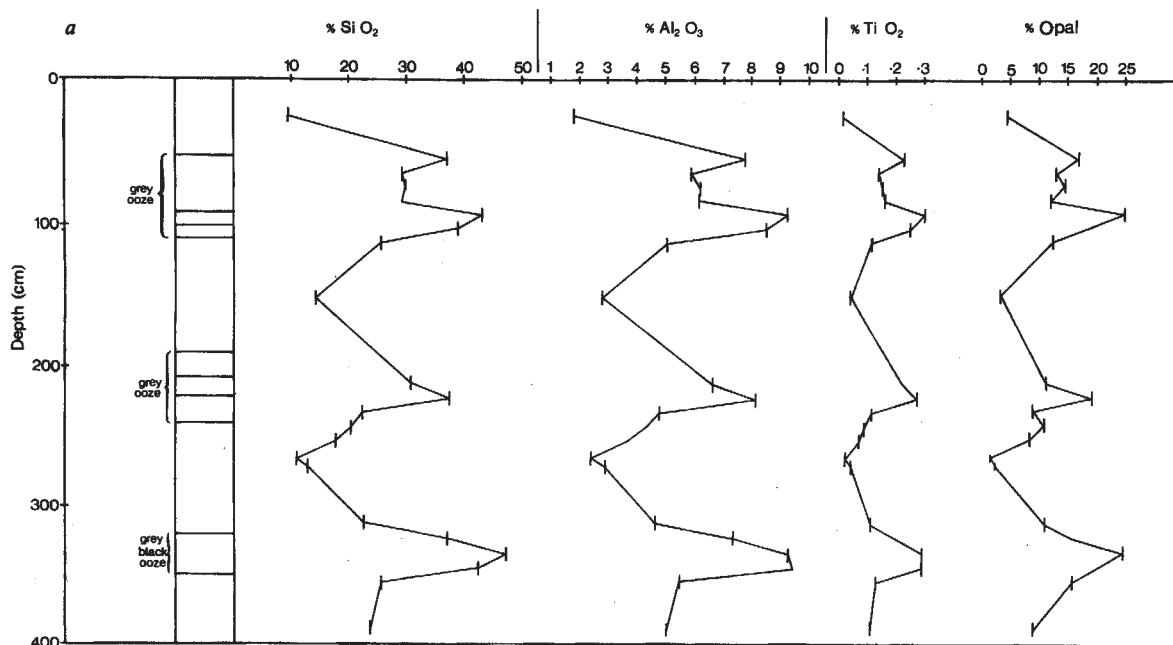
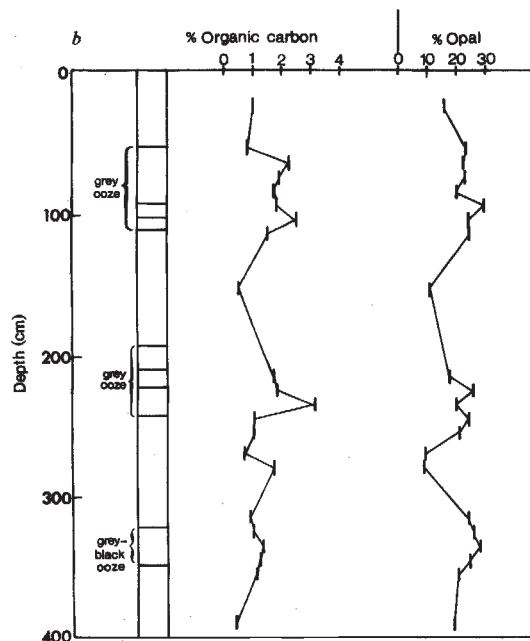


Fig. 3 a, Basic mineralogical composition (% dry weight of sediment). b, Organic carbon and opaline silica data expressed on a carbonate-free basis.



in this area and such sedimentary sites would be expected to receive a considerable contribution of organic matter from terrigenous sources resulting in greatly more negative $\delta^{13}\text{C}$ values.

Preliminary analyses on the component lipids from the dark band within the most recent ooze (97–102 cm) show a fatty acid profile dominated by C_{14} – C_{18} even chain acids (>85% total fatty acids). Long chain fatty acids (C_{26} – C_{30}), which are typical organic components of most terrigenous inputs, were only minor components (<6% total fatty acids). Such a fatty acid profile is taken to indicate that marine phytoplankton dominated the organic input during this period of the core's history with a minimal input of terrigenous organic material^{23,24}. There was a considerable monounsaturated fatty acid component (11–12% total fatty acids) which is somewhat surprising for a sediment sample at least 30–40,000 yr old. We believe that for such good preservation of labile, unsaturated lipid the original organic source material must have been deposited rapidly in a relatively intact state.

Studies on some of the organic-rich oozes which underlie the present day highly productive areas of the ocean (the upwelling areas off Namibia and Peru) have indicated that in the relatively shallow continental shelf and slope areas the organic material in the sediments originates mainly from the rapid sedimentation of phytoplankton blooms^{21,23–28}. It has been suggested that the sedimentation occurs in a series of discrete 'events', giving large pulses of biological material which sink rapidly blanketing the underlying sediment surface²⁵ and ensuring the burial of large quantities of the organic debris. In such conditions two processes occur which are thought to aid the preservation of the sedimented organic material. First, an increased flux of organic compounds to a sediment–water interface stimulates microbial activity with a concomitant development of anoxic conditions in the interfacial sediments. Thence bacterial degradation will be limited to all but the anaerobic species and auto-oxidative breakdown will be much reduced. Second, high molecular weight humic compounds are rapidly formed from the natural product organic molecules present in phytoplankton detrital material^{26,29,30}. These high molecular weight moieties will be more resistant to chemical and biological attack than the initial natural product molecules.

Work on phytoplankton blooms occurring in experimental enclosed ecosystems has largely supported these hypotheses^{29,30}. However, they only relate to geochemical processes which are

Table 1 ^{14}C dating and $\delta^{13}\text{C}$ values

Sample horizon (depth in core) (cm)	Age based on carbonate ^{14}C (yr)	Average accumulation rates (cm kyr ⁻¹) based on carbonate ^{14}C	Age based on organic ^{14}C (yr)	Average accumulation rates (cm kyr ⁻¹) based on organic ^{14}C	$\delta^{13}\text{C}$ (‰)
20–30	5,100 ± 90	2.95			
50–60	15,260 ± 230				
60–70	27,720 ⁺⁶³⁰ ₋₅₉₀	0.80	17,320 ⁺⁸¹⁰ ₋₈₃₀	3.23	-20.1
70–80	30,640 ⁺⁹²⁰ ₋₈₃₀	3.42	20,460 ⁺⁹³⁰ ₋₈₃₀		-19.4
80–90	37,320 ^{+2,290} ₋₁₇₈₀	1.50			
90–100	>33,720				
100–110	>37,400				

thought to occur in marine water columns of, at most, a few hundred metres.

The development of stagnant hydrographical conditions have normally been invoked as the primary process^{1-10,31} for the preservation of organic material in sediments underlying water columns of several thousands of metres depth. However, peaks in the organic carbon profiles of recent deep-water sediments (2,700–3,500 m) from the eastern equatorial Pacific¹⁵ has been taken as evidence for periods of significantly increased productivity in the area during the last glacial maximum. The sediment under present study is from a considerably deeper core site (4,735 m) in the middle of a flat abyssal plain interrupted only by a few isolated sea mounts. It is very difficult to imagine that stagnant conditions have prevailed in the overlying water columns at this site during the history of the core (~300,000 yr). Whilst conclusive evidence is not available to prove this point, there is strong evidence to support the hypothesis that the

deep-water, organic-rich ooze deposits found in this core from the Guinea Basin were a direct result of periods of increased productivity in the surface layers of the overlying oceanic water column. First, the core mineralogy shows that the ooze deposits were associated with a considerable increase in the input of biogenic silica. Second, the most recent peak in organic carbon corresponds to a period of raised sedimentation. Third, the stratigraphical analysis, ¹⁴C carbonate age profile, ¹³δC measurements, quartz data and fatty acid analyses all show no major turbidite influence in the core. Fourth, the ¹³δC and fatty acid data indicate that the organic matter in the most recent ooze deposits mostly originated in marine plankton.

The recent report of a seasonal pulse of organic detrital material to bathyal and abyssal depths being derived directly from the surface primary production³² supports this theory.

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Isotope studies of insular phosphates explain atoll phosphatization

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Carbonate apatite¹ with the general formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{F}, \text{OH})_2$ is a major component of the phosphate deposits that occur on numerous coral reef islands²⁻⁶. Such insular phosphates are generally accepted to be the result of chemical interaction between solutions leaching seabird guano and the underlying reef limestone^{2,7}. Details of this process, however, and the specific environmental conditions which promote it, are not yet fully understood⁸. We present here oxygen and carbon isotope data from carbonate apatites which elucidate the process of phosphogenesis on the Pacific atolls and the palaeoclimatic conditions in which it occurs. We suggest that there were drier climates in the tropics during the Quaternary ice ages associated with an extension of equatorial upwelling in the Pacific.

In the expectation that the oxygen and carbon isotopic composition of CO_2 sources of structural carbonate in apatite may leave a characteristic fingerprint of the depositional environment^{9,10}, we have initiated an exploratory study of oxygen and carbon isotope abundances of insular phosphates from coral reef islands in the tropical Pacific (Fig. 1). Samples from Ebon Island (4°35' N, 168°43' E) and Nauru (0°25' S, 166°57' E) (see Table 1) were chosen as representative of minor phosphate rock deposits on low-lying atolls and major deposits on high islands

respectively. Two samples from Ocean Island (0°53' S, 169°35' E) and Makatea (15°50' S, 148°13' W) are also included to augment the representation of high oceanic islands capped by phosphates and separated by wide stretches of ocean (Table 1, Fig. 1). The phosphate from Ebon Island is typical of atoll phosphate rock, or "crust guano"^{2,11}. The material consists of coral rubble, mainly coral aragonite, bound together with thinly laminated colophane (cryptocrystalline apatite) cement, thought to be a primary precipitate which forms when phosphatic solutions leached from guano first come into contact with the limestone substrate. Minor aragonite micritic cement coexists with the apatite in the fine laminae. The phosphates from the high-lying atolls are of two distinct types (see Table 1). The first are typical of the unconsolidated phosphate ore filling the depressions between pinnacles (karst remnants). These samples are fine to medium-size pisolitic and oolitic pellets with a markedly concretionary structure and are thought to be formed by rapid interaction of the solutions leached from the guano with the directly underlying coral-sand⁷. The second are phosphatized reef rocks *in situ*, including: (1) pure apatite cast of reef-coral where the coral carbonate skeleton has been completely removed by subsequent dissolution; (2) banded phosphate layers overlying pinnacles; (3) translucent (agate-like) phosphate called nauruite² occurring in depressions and cracks of the pinnacles. Materials produced from the capping of phosphate on Ocean Island, similar to the two types above, are termed by Owen⁷ as incoherent and coherent phosphates respectively. We also took samples from the black phosphatic soil which covers the phosphate ore on Nauru with a thin layer. To place constraints on the timing of the apatite emplacement in the respective deposits, U-series age estimates have been used¹².

Experimental procedures were similar to those described by Kolodny and Kaplan⁹, but were designed to permit separate analysis of CO_2 derived from the apatite and associated components, that is carbonate and organic matter. Accordingly, any carbonate coexisting with the apatite was selectively removed in dilute acetic acid¹³. The remaining apatite, verified by X-ray

diffraction measurements to be free of any associated carbonate, was then dissolved at 25 °C in 100% H₃PO₄ for 72 h. The isotopic fractionation of oxygen in the evolved CO₂ was assumed to be the same as calcite⁹. The organic matter residue was filtered on silicon paper and combusted at 900 °C in pre-evacuated quartz tubes in the presence of CuO and silver wire¹⁴. The aragonite cement from the Ebon phosphate was chipped off under a microscope and analysed separately. The CO₂ evolved from the separate fractions was analysed in a gas-source mass spectrometer for ¹⁸O/¹⁶O and/or ¹³C/¹²C isotope ratios following standard procedures¹⁵. The results reported according to the conventional 'δ' notation are shown in Table 1. The δ¹⁸O and the δ¹³C values from Table 1 are plotted in Fig. 2 together with those from known or likely sources for the apatite-CO₂.

These data can be interpreted as follows: The δ¹⁸O value of -1.5‰ for the apatite-CO₂ of Ebon Island phosphate closely approximates the δ¹⁸O value of -1.6‰ in the coexisting aragonite cement and is compatible with a CO₂ source in isotopic equilibrium with seawater¹⁶⁻¹⁸. The difference of δ¹³C observed between the aragonite and the apatite-CO₂ (2.5‰ and 0.7‰ respectively, Table 1, Fig. 2) implies that ~8% of the CO₂ could be attributed to contamination of the inorganic carbon pool by aerobic degradation products of guano. This interpretation is supported by the δ¹³C of the organic matter associated with the apatite (-23.5‰, Table 1) which is near that of modern guano (δ¹³C = -19.5‰) from Heron Island—a typical lagoonal platform near the southern end of the Great Barrier Reef.

Although neither Ebon nor Nauru presently support seabird colonies, the high contents (up to 9%, Table 1) of organic matter associated with the apatite from Ebon is consistent with our interpretation of a guano source, as no other mechanism would introduce such high carbon contents. The carbon-isotopic composition and the high concentration of the phosphate organic fraction thus provide direct evidence for seabird guano as the most likely and direct precursor of insular phosphates in accordance with Hutchinson's proposals^{2,19}.

Involvement of seawater during apatite formation on Ebon Island, implied by the oxygen isotope data, is independently supported by the relatively high fluorine content of the apatite fraction, because neither guano nor reef limestone are adequate sources for this element¹². In fact, the wide range of fluorine content exhibited by different insular phosphates² has been attributed to variable exposure of insular apatites to sea spray or seawash²⁰ which in the case of low island deposits is not difficult to envisage.

By contrast, the distinctly light δ¹⁸O and δ¹³C yielded by the phosphates from Nauru (Table 1) may indicate apatite deposition from meteoric waters presumably following emergence of Nauru more than 200,000 yr ago. This suggestion is supported by (1) the proximity of the δ¹⁸O and δ¹³C values to the field of coral-reef limestones recrystallized in subaerial environments on Pacific islands (Fig. 2); (2) petrographic evidence which indicates that some of the coherent apatites formed as casts that preserve coral textures and that subsequently the coral dissolved presumably on subaerial exposure; and (3) geological evidence² which indicates that phosphatization of the atoll evidently occurred when Nauru was even more elevated than it is today. The isotopic compositions of the apatites from Ocean Island and Makatea, which are within the range of values reported for the apatites from Nauru, suggest a strong similarity between the depositional environments of these phosphate deposits. Two additional points require further discussion.

First, the extreme light δ¹³C compositions of the apatite-CO₂ from Nauru, averaging -12‰, is consistent with a source of carbon in the meteoric water formed by roughly equal amounts of CO₂ generated by guano aerobic decay (δ¹³C = -19.5‰) and dissolution of a reef limestone substrate of δ¹³C = -4.5‰ (ref. 21). Variability in the observed δ¹³C compositions of the apatites could reflect either departure from a ratio of 1 between the two CO₂ endmember sources and/or limestone substrates of variable δ¹³C compositions (Fig. 2).

Table 1 Experimental data and stable isotope results of insular phosphates and seabird guano

Sample no. and island location	Field occurrence*	Source of CO ₂ analysed	Inferred age† (yr)	Weight % CO ₂	Weight % OM	δ ¹⁸ O‡ (‰ PDB)	δ ¹³ C‡ (‰ PDB)
Ebon							
EB 1		Total phosphatic rock		7.4		-1.72	1.51
EB 2		Aragonite cement				-1.63	2.51
EB 3	lc	Apatite	4,000 ± 300	1.0	9	-1.46	0.74
EB 4/1		Organic residue					-23.29
EB 4/2		Organic residue					-23.76
Nauru							
NR 80-5	sp	Apatite	>200,000	1.8	Trace	-6.05	-11.39
NRU-5	sp	Apatite		1.9	Trace	-6.19	-11.85
NRU-3	sp	Apatite		1.3	Trace	-5.97	-12.02
NRU-4	sp	Apatite		1.0	Trace	-5.84	-10.99
NRU-18	sp	Apatite		2.0	Trace	-6.88	-12.42
NRU-19	sp	Apatite		0.8	Trace	-6.41	-11.32
NRU-20	sp	Apatite		1.2	Trace	-6.50	-13.46
NR 3/1	rc	Apatite	>200,000	2.0	Trace	-6.03	-8.53
NR 80-9A	bl	Apatite	>200,000	3.8	Trace	-5.28	-8.50
NR 80-8	na	Apatite	>200,000	3.4	Trace	-6.28	-10.93
NRU-1	bs	Apatite		1.3		-6.10	-11.26
Ocean island							
OC-2	sp	Apatite	>200,000	1.3	Trace	-5.58	-9.39
Makatea							
MK-1	sp	Apatite	>200,000	2.2	Trace	-4.40	-10.89
Heron							
HG 1/1		Guano	Modern		98		-19.74
HG 1/2		Guano	Modern		98		-19.54

* lc, Laminated colophane; sp, unconsolidated phosphate ore of sand-sized pellets; rc, cast of reef coral; bl, banded phosphate layers on top of pinnacle; na, translucent phosphate (naurite); bs, black phosphatic soil.

† Based on U-series age determinations of apatite fraction (ref. 12 and H.H.V., unpublished).

‡ The overall error (1σ), based on standards analysis, is estimated to be ±1‰.

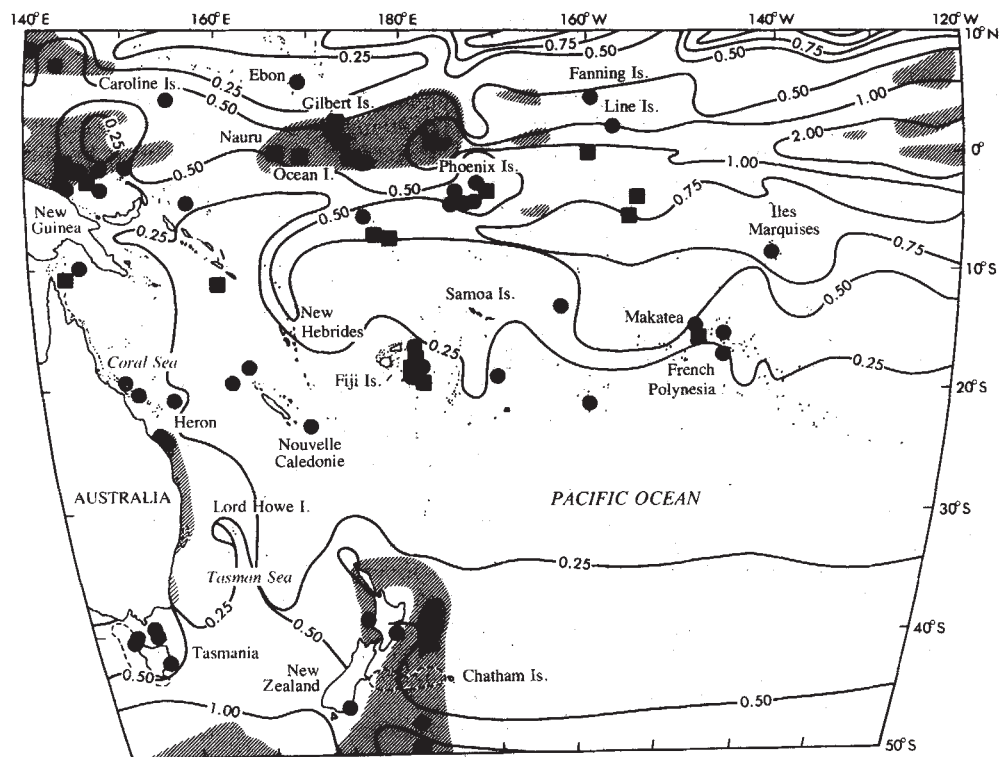


Fig. 1 Distribution of guano phosphate deposits in the South Pacific Region and pertinent ocean chemistry and biology data (after ref. 32).

- 0.75 Phosphate concentration at 100m $\mu\text{g atom P/litre}$
- Known phosphate nodule accumulations
- Major guano deposits
- Minor guano deposits
- Zooplankton abundance over $100\text{cm}^3/1000\text{m}^3$
- Zooplankton abundance over $500\text{cm}^3/1000\text{m}^3$

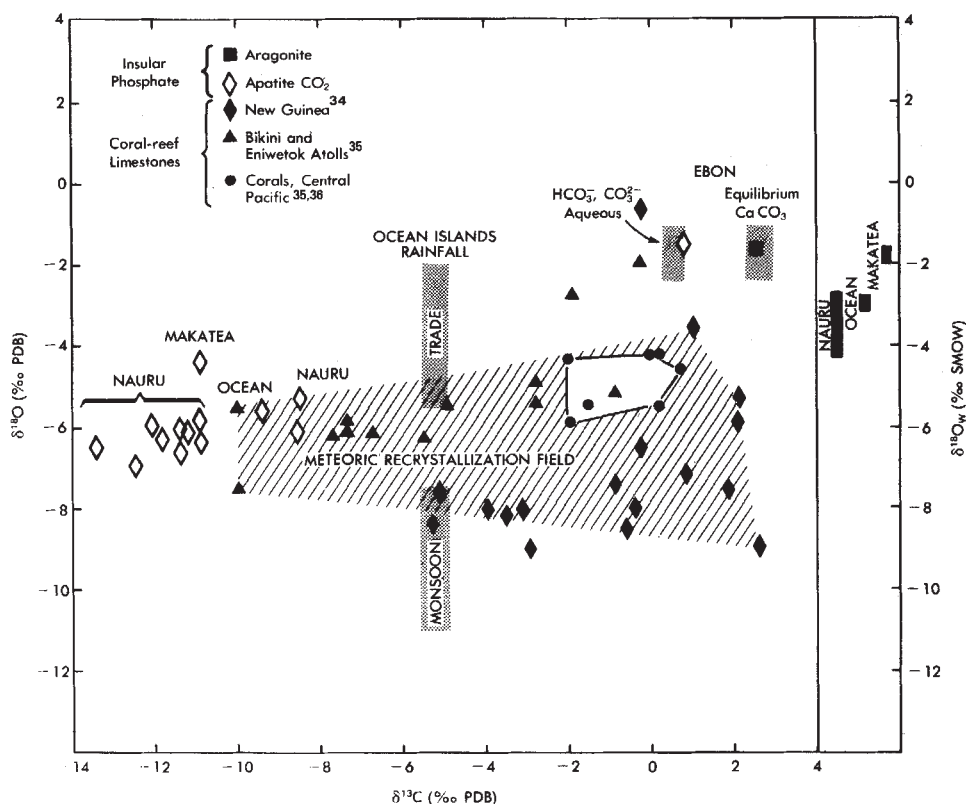


Fig. 2 $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values of phosphatic material from low and high islands (Ebon and Nauru, Ocean Island, Makatea respectively) and the isotopic ranges of possible inorganic CO_2 sources. On the right column are the estimated $\delta^{18}\text{O}$ ranges of the palaeowaters from which the respective phosphates were deposited. Note that carbonate and phosphate $\delta^{18}\text{O}$ values are reported relative to the PDB standard while water is relative to SMOW standard. The relationship between the two reference standards is given by the expression³³: $\delta^{18}\text{O}_{\text{PDB}} = 0.97\delta^{18}\text{O}_{\text{SMOW}} - 29.94$.

Second, groundwater which is abundant during subaerial diagenesis is a large reservoir and buffers the $\delta^{18}\text{O}$ of chemical precipitates in the vadose zone^{21,22}. With one qualification to be discussed below, the narrow $\delta^{18}\text{O}$ range yielded by the subaerial phosphates reported here probably reflects the narrow $\delta^{18}\text{O}$ range of local rainfall during diagenesis²². In this context, the ^{18}O -enrichment of 2.5‰ observed in the apatite from Makatea relative to the apatites from Nauru (Fig. 2) is consistent with the documented $\delta^{18}\text{O}$ differences between the western and eastern tropical Pacific rainfalls²³.

If the apatite- CO_2 formed in isotopic equilibrium with the meteoric water, then the oxygen isotope composition of the phosphatizing solutions can be estimated using an ambient temperature of 28 °C, the present mean monsoonal temperature on tropical Pacific islands, and the calcite geothermometer²⁴. The latter has been shown to be indistinguishable from the apatite- CO_2 geothermometer²⁵. Viewed this way, $\delta^{18}\text{O}$ values of the phosphatizing solutions range between -4.3 to -2.7‰ SMOW on Nauru and Ocean Island and -1.8‰ SMOW on Makatea (Fig. 2).

Studies have shown that $\delta^{18}\text{O}$ values of groundwater reflect the isotopic composition of precipitation during the season of maximum infiltration²⁶. Thus abundant monsoonal rainfalls, unusually depleted in ^{18}O relative to the trade precipitation (Fig. 2), must provide the main source of meteoric water at tropical Pacific sites affected by the monsoonal circulation^{27,28}. For example, the ^{18}O -depleted meteoric water is well imprinted in the $\delta^{18}\text{O}$ compositions of late Pleistocene coral-reef limestones from New Guinea shown in Fig. 2. As isotopic modelling²⁹ indicates that Nauru and Ocean Island presently pertain to the same province of light $\delta^{18}\text{O}$ rainfall as New Guinea ($\delta^{18}\text{O} = -6$ to -8 ‰ SMOW), this reasoning would suggest that phosphates on these islands were formed from meteoric waters with ^{18}O contents appreciably different from present precipitation in the region. The apparent ^{18}O -enrichment may be attributed to the effect of monsoon failure, increased evaporation, or both, and in either case mean that major phosphate deposits of Pleistocene or earlier ages on high islands were formed in much drier climatic conditions than those now prevailing.

According to Hutchinson^{2,19}, the distribution pattern of islands with modern guano deposits suggests that organic productivity caused by upwelling, coupled with low rainfall, favour the net accumulation of guano and hence should provide an adequate source of phosphorus for the formation of insular phosphate deposits (Fig. 1). However, the distribution pattern of ancient insular phosphate is quite different from that of modern guano deposits and cannot readily be explained in terms of present climatic conditions.

The results of this study tentatively support Hutchinson's explanations of these differences, namely that they are related to shifts in frequency and abundance of tropical rainfall during the ice ages—factors which also affect the temporal and spatial distribution of guano phosphate deposits. Our suggestion of drier climates in the tropics during the Quaternary ice ages is quite consistent with an intensified Hadley circulation³⁰ and a strong development and extension of equatorial upwelling in the Pacific³¹.

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An extraordinary peat-forming community on the Falkland Islands

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Most of Beauchêne Island in the South Atlantic is covered by tussac, the tussock-forming grass *Poa flabellata* (Lam.) Rasp., which has produced a deep accumulation of exceptionally dense peat during ~12,500 yr. The basal peat is lignitic, yet it is several hundred times too young to be a true lignite. During an ecological survey of the island in December 1980¹, one of us (R.I.L.S.) sampled an 11-m high peat face. The age against depth profile in the peat is consistent with a constant proportional rate of decay of $1.1\text{--}2.2 \times 10^{-4} \text{ yr}^{-1}$ and a constant rate of addition of dry matter to the peat of $430\text{--}720 \text{ g m}^{-2} \text{ yr}^{-1}$. This rate of decay is within the range recorded for peats in corresponding latitudes in the Northern Hemisphere, but the rate of addition of dry matter is about 10 times as great. This is not easy to accommodate within current hypotheses about peat formation. An unusual combination of biological, physical and chemical circumstances may be the cause. As the island is difficult to visit and no more information can be obtained in the near future, we now report these results, incomplete though they are.

Beauchêne Island^{1,2} (lat. 52°54' S, long. 59°04' W) is the most southerly and isolated of the Falkland Islands in the South Atlantic Ocean. It is ~187 hectares in area, rises to 82 m altitude and is 60% covered by continuous closed tussac grassland. Individual plants, at a density of 600–700 per hectare, reach 3.5 m in height, including a peaty pedestal 1.0–1.5 m tall, and 2 m in diameter, and have a canopy up to 4.5 m across; a skirt of dead foliage extends from the side of the pedestal and interlaces with the skirts of adjacent plants. This is an almost truly monospecific plant community with almost no ground flora.

A further 35 hectares are occupied by dense mixed colonies of about 300,000 pairs of rockhopper penguins (*Eudyptes chrysocome*) and 160,000 pairs of black-browed albatrosses (*Diomedea melanophrys*).

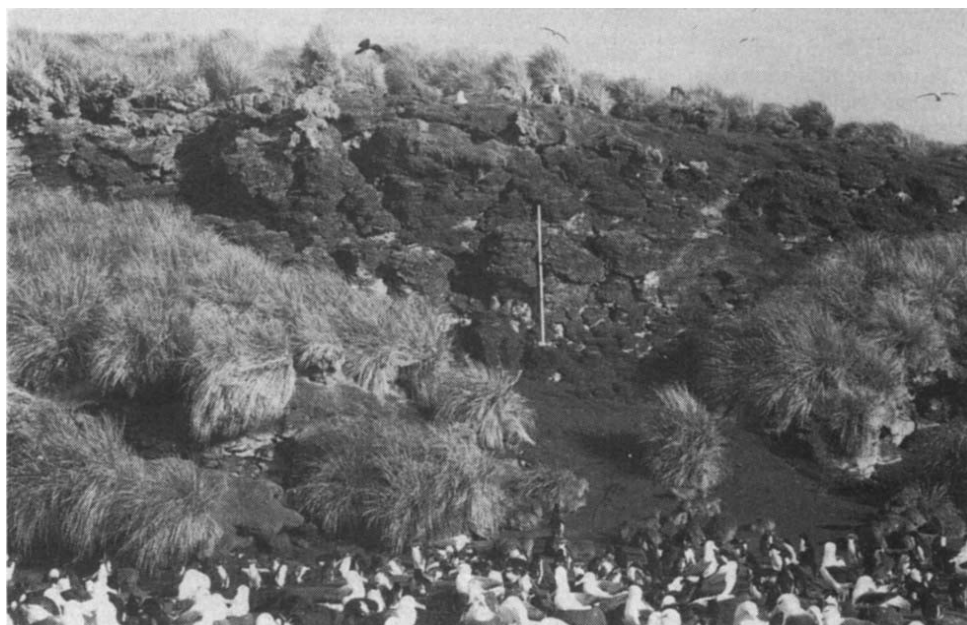


Fig. 1 An eroded cliff in the western edge of peat formed by tussac (*Poa flabellata*) grassland on Beauchêne Island in the South Atlantic. The birds in the foreground are part of a breeding colony of rockhopper penguins and black-browed albatrosses. This is the site where our samples were taken. The white stake is 2.80 m long and the depth of peat from the base of the grass tussocks to the bedrock is 11.3 m. At the north-east edge of the island is a similar cliff in 5 m of peat.

The grassland grows on a deep mantle of peat composed of *Poa flabellata* litter. Along about one-third of the main bird colony the western edge of the tussac grassland is eroded into a steep and often near-vertical peat cliff (Fig. 1) along which the maximum recorded depth was 13.3 m.

Beauchêne Island has a cool temperate oceanic climate similar to, but probably cooler, drier and windier than, that of Port Stanley (Fig. 2) on the east side of East Falkland Island. It is similar to that of Tierra del Fuego. Compared with Moor House in the North Pennines of England, which has extensive blanket bog, the temperature regime is more equable with little frost, and the precipitation is much lower. Other regions of the Northern Hemisphere, such as Ireland, have extensive peat bogs with as equable a temperature regime as Beauchêne Island but even larger precipitation than Moor House. Yet others, such as continental Europe, North America and north Eurasia, have peat bogs with a much less equable temperature regime and similar precipitation.

The concentrations of inorganic species in leaves of one *Poa flabellata* plant and in three samples of surface peat from Beauchêne Island were determined². These values are compared with some for North German surface peats (Table 1). The influence of sea spray is clear in the values for Na, but not in Mg. The cation exchange and chelating capacities of the Beauchêne Island peat are smaller than those of the North German peats, and this may reduce selectivity for higher valence ions. The absence of soil dust may partly account for the lower values of Ca, Fe and Al: the island rock is quartzite. Inorganic and total N are high in the Beauchêne Island peat, probably a consequence of the close proximity of so many nesting birds, but PO₄ concentrations are not very high. In the live plant there are higher concentrations (than in the peat) of NH₄ and especially of K. In general there seem to be no obvious deficiencies in the live plants, but the concentration of K in the peat may perhaps limit the activities of microorganisms, particularly at pH 3.7 which now prevails near the surface (Fig. 3).

The physical properties of the peat are unusual. The water content was measured on samples of peat stored for about 2 weeks in sealed polythene bags. The dry bulk density of air-dried peat was measured much later, but the results showed that during air-drying the peat had shrunk considerably. The dry bulk density of fresh peat was therefore measured indirectly by two methods. A sample was heated in water at 98 °C for 24 h. The peat was strongly coherent. Its volume was determined by displacement. The surface was blotted and the rehydrated fresh mass measured. Then the sample was put into a 'specific gravity' bottle whose mass was measured. Finally the sample was dried

at 105 °C for 24 h and its mass measured. From the 'specific gravity' measurement the dry matter density, and then the volume of dry matter, were calculated. This, together with the fresh and dry mass, gave the wet volume of the rehydrated peat. The results agreed to within 5% with the direct measurements of volume. Next it was assumed that the difference in water content of the rehydrated sample and of the original wet peat gave a direct measure of the loss of volume from the peat in the fresh state and so, finally, the dry bulk density. The second method used the fact that the density of the dry matter (not the dry bulk density) changed approximately exponentially from 1.44 g cm⁻³ at 75 cm depth to 1.62 at 1,100 cm depth. With this information and the water content of the original wet peat, assumed water saturated, the minimum value of the dry bulk density was calculated. For nine samples below 125 cm deep, this calculation gave results within 3% of those obtained by the first method. This close agreement probably indicates that the dry bulk density values are sufficiently accurate and that the peat below 125 cm depth is water-saturated and hence probably anaerobic. The first method gave higher values than the second for the two topmost samples: 0.29 compared with 0.25 g cm⁻³ at 75 cm; 0.34 compared with 0.30 at 125 cm. This is consistent with some gas in the fresh peat at these depths, or with bias in the first method applied to these markedly less-coherent peats. Whatever the explanation it does not seriously affect the use of the dry bulk density values for the assessment of the peat

Table 1 Concentration of some inorganic species (μmol g⁻¹) and loss on ignition (%) on a dry mass basis

	Beauchêne Island <i>Poa flabellata</i> green leaf mid-lamina	Surface tussac peat: range of three values	North Germany ⁹ Surface peat inter- quartile range
Na	160	120–150	2–10
K	490	2–10	10–60
$\frac{1}{2}$ Ca	60	110–490	200–500
$\frac{1}{2}$ Mg	70	83–130	160–800
$\frac{1}{3}$ Fe	10	80–90	100–300
$\frac{1}{3}$ Al	10	80–90	170–1,100
PO ₄ – P	180	6–120	30–650
NO ₂ + NO ₃ – N	0.5	0.8–5.4	<0.01–0.01
NH ₄ – N	240	9–27	1–15
Total – N	1,700	2,500–4,900	700–1,800
Loss on ignition	95	78–85	50–95

accumulation process (Fig. 3). In the absence of measurements of redox potential, however, we do not know the extent to which the surface layers are aerobic, and this is crucial in any attempt to understand the decay processes³.

The bulk density of dry matter is unusually high for bog peat and the loss in mass on ignition was high (89–95%) so the high bulk density cannot be attributed to the presence of large amounts of inorganic matter. The bottom 150 cm of the peat are lignitic. Where this peat is exposed to the air it becomes black, rock-like, and breaks with a conchoidal fracture². The field water content of this material is ~30% (of the dry mass) but about 1 m from the peat face the water content is ~200% and the peat has a very hard cheesy texture. True lignite is chemically altered by geothermal heat and is >10⁶ yr old, yet ¹⁴C dating shows that the lignitic peat is younger by several

hundred-fold. The compressive stress at the base of a water-saturated peat may be quite small, because the effective weight is produced only by that part of the dry matter with density greater than that of water. Thus for a 10-m depth of water-saturated peat of dry bulk density 0.3 g cm⁻³ and with dry matter density 1.5 g cm⁻³, the pressure will be (1.5–1.0) × 0.3 × 1,000 = 150 g cm⁻².

The ¹⁴C-age of 10 samples spaced down the peat profile was measured (Fig. 3) and is consistent with a rate of addition of dry matter of $p = 520\text{--}720$ (95% confidence limits) g m⁻² yr⁻¹ and of proportional rate of decay of $\alpha = 1.2 \times 10^{-4}\text{--}2.2 \times 10^{-4}$ yr⁻¹. The asymptotic limiting depth, p/α , is 360 g cm⁻² (there is ~330 g cm⁻² at present). If the dry bulk density of this matter were ~0.35 g cm⁻³ then the limiting depth would be ~14 m. The position of the lowest two points may be seriously inaccurate because of the ¹⁴C calibration extrapolation, and because they are in the lignitic peat which may affect the assumption of no more than two constant processes. So the topmost eight points alone were fitted too. They gave $p = 430\text{--}630$ g m⁻² yr⁻¹, $\alpha = 1.1 \times 10^{-4}\text{--}1.7 \times 10^{-4}$ yr⁻¹, and an asymptotic depth of 15 m.

These results are similar to those found for Fennoscandian peats⁴ in that (1) depth, as cumulative mass, versus age curves appear to fit the single component, constant rate of addition, constant proportional rate of decay hypothesis surprisingly well; (2) independent of assumptions about the rate of decay, the rate of addition of dry matter has been nearly constant over thousands of years in spite of probable climatic changes; (3) the rate of decay of Beauchêne Island peat is within the range (0.7–5) × 10⁻⁴ yr⁻¹ for Fennoscandian peats; (4) the rate of addition, p , of dry matter to Beauchêne Island peat is about 10 times as great as the rate (35–78 g m⁻² yr⁻¹) for Fennoscandian peats⁴ (Fig. 3). The quoted Fennoscandian values of p are the rate of addition to the anaerobic peat and are ~10% of the net productivity of the vegetation there. If the same were true on Beauchêne Island then net productivity would be about 5,500 g m⁻² yr⁻¹ which is an implausibly (but not impossibly) large value: the relative growth rate of *Poa flabellata* tillers in experiments^{5,6} over the 24-week austral summer was 0.13 g g⁻¹, and a capital of 240 g m⁻² invested with this growth rate would be sufficient to produce 5,500 g m⁻² in 24 weeks. The dry green biomass in a site close to a seal wallow on South Georgia was 16,000 g m⁻².

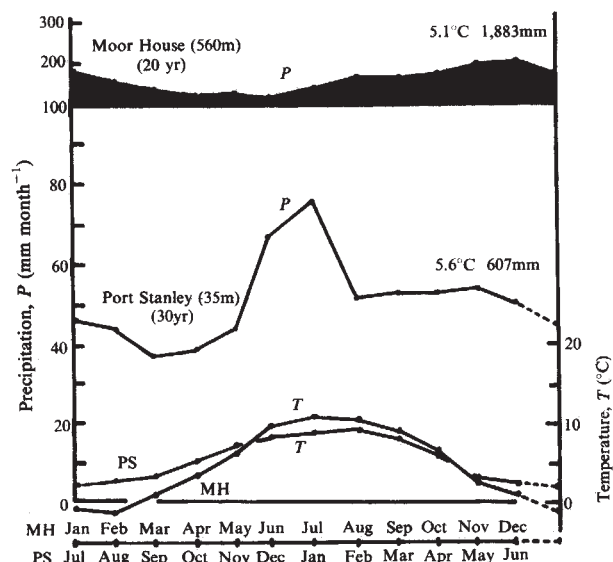
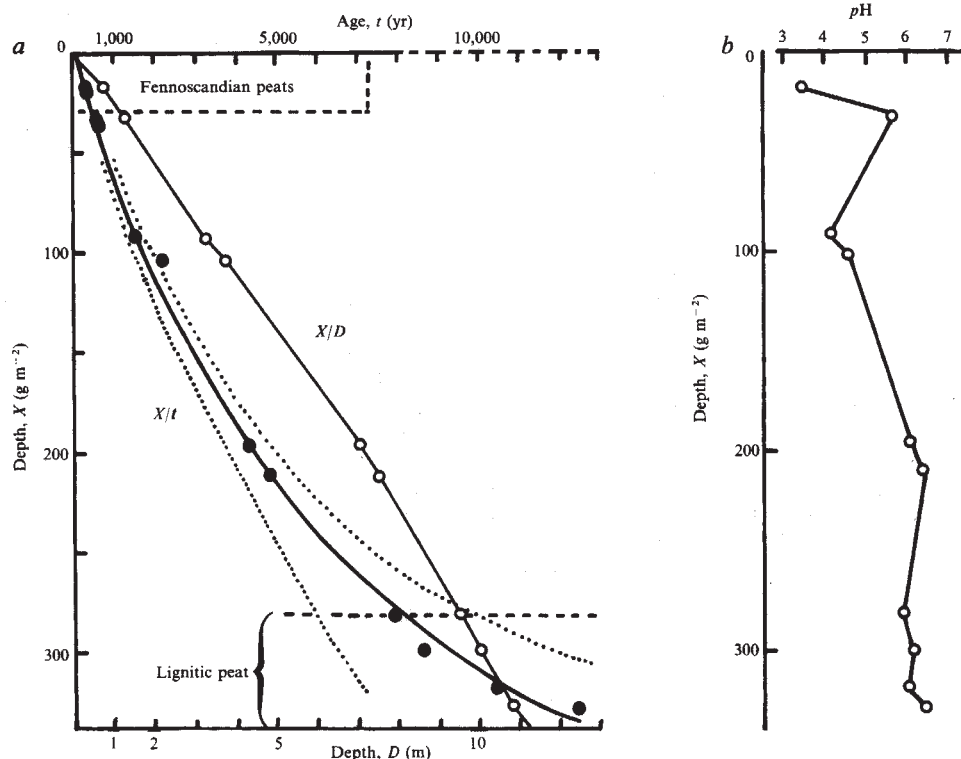


Fig. 2 Klimadiagramms (superimposed) for Port Stanley (PS), East Falkland Island and Moor House (MH), North Pennines, England.

Fig. 3 a, Age, t , against depth as cumulative mass, X , and depth as distance, D , against depth as cumulative mass for a *Poa flabellata* peat profile on Beauchêne Island, South Atlantic. The 'true' age, t , of samples with ¹⁴C age up to 6,500 yr was calculated using a dendrochronological calibration¹⁰. For the oldest thousand years of this calibration the deviation between ¹⁴C and dendrochronological age is approximately constant and this deviation has been assumed to hold for older samples also. On this plot a constant rate, p , of addition of plant mass to the peat would give a straight line, and if there is decay in the peat a hollow curve results^{3,4}. If the proportional rate of decay, α , is constant, and the peat is formed of a single component, then $X = (p/\alpha)(1 - e^{-\alpha t})$. The fitted curve⁴ is for $p = 620$ g m⁻² yr⁻¹ and $\alpha = 0.00017$ yr⁻¹. The estimated value of p is largely determined by the general slope of the line, and of α by the concavity. Dotted lines show 95% confidence limits. The box at the top left shows the approximate region occupied by peats in Fennoscandia⁴. b, pH against depth.



It is difficult to account for the Beauchêne Island peat. The concentration of inorganic elements in the plants reveals little about what limits the productivity of *Poa flabellata*. The species favours organic soils and is highly tolerant of sea-spray and biotic disturbance. Its luxuriance and productivity are enhanced wherever there are breeding populations of seals and seabirds. It is not known why the plant matter does not decay as rapidly as it forms. Populations of bacteria, yeasts and other fungi in the peat are high². The temperature is not so low as to restrict decay in general. At the much colder sub-Antarctic South Georgia tussac peat accumulates to several metres in depth⁷ yet decomposition of other organic matter proceeds quite rapidly⁸. Why then does peat accumulate on Beauchêne Island at all? And why is the rate of input to it so high at the same time as the decay rate is low? Part of the reason must be that the large input of volatilized nitrogenous compounds from the adjacent massive seabird colonies promotes rapid growth of the grass. In contrast, decomposition may be slow because of the low rainfall and high evaporation rates, caused by the almost perpetual strong winds, which together maintain a fairly dry substrate moisture regime in the surface layer of peat. In Arctic tundra peats a moisture content <300% inhibits microbial activity and decomposition rates. The water content of surface peat on Beauchêne Island is 250–350%. Potassium may also be deficient. The Beauchêne Island *Poa flabellata* peat raises fundamental issues about how peat forms, and would repay detailed study.

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Heredity and familial environment in intelligence and educational level—a sibling study

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Although it is well established that family members resemble each other in intelligence¹, the extent to which this results from either shared genes or a shared environment remains controversial², perhaps especially since the relevant evidence presented by Burt has been shown probably to have been fabricated³. The influence of heredity and familial environment may be distinguished by studying adoptees. Here we present correlations in intelligence and educational level between genetically related pairs of adult adoptees who have been reared separately, and, conversely, between genetically unrelated pairs of adult adoptees who have been reared together. We are unaware of any previous study of adults which has reported on both of these types of relationship. The results for intelligence conform closely to what would be predicted by a simple polygenic model of genetic transmission whereas those for educational attainment imply both genetic and familial environmental components.

From a Danish adoption register^{4,5} we have identified four groups of sibling pairs. The first three groups comprise genetically related pairs who were adopted and reared apart in separate

families: full siblings reared apart (FA, 34 pairs), maternal half-siblings reared apart (MA, 52 pairs) and paternal half-siblings reared apart (PA, 41 pairs). The fourth group comprises genetically unrelated pairs who were adopted and reared together in the same families (UT, 27 pairs). All of these adoptees were male, born between 1938 and 1947. Their median age at transfer to their adoptive homes was 5 months, and 86% had been transferred by 2 years of age.

Intelligence and education data for these adoptees were obtained from draft board records. Danish conscription law requires males, on attaining the age of 18 (or shortly thereafter, and not later than 26 years old), to appear before a draft board. A medical examination is performed, educational level (EL) is recorded on a 9-point scale from 1 = minimum years of grade school (that is, 7) to 9 = university degree, and an intelligence test, the Børge Prien Prøve (BPP)⁶, is administered.

The BPP is a 45-min group test consisting of four subtests covering broad areas of cognitive functioning: letter matrices (similar to Raven's progressive matrices), verbal analogies, number series, and geometric figure analysis. Each subtest comprises approximately 20 items arranged in order of increasing difficulty. The BPP score is the total number of correct answers.

BPP and EL scores, together with height, were traced in the draft board archives for 28 FA pairs, 34 MA pairs, 30 PA pairs and 24 UT pairs.

We have also identified a fifth group comprising 73 pairs of non-adopted brothers, that is, full siblings reared together by their biological parents (FT). These pairs, for whom height and BPP and EL scores were also available, are derived from a large sample of males used in another study⁷ and differ somewhat from the adoptees. They were born between 1944 and 1947, they were almost all reared in Copenhagen rather than, as were the adoptees, distributed across Denmark, and they were at least 184 cm tall.

Quantitative predictions from a familial environmental or genetic hypothesis, regarding the degree of resemblance to be expected between such pairs of adopted and non-adopted siblings on any given measure, will to some extent depend on the parameters included and on the assumptions made. Heuristically, however, the following simplification may be proposed in terms of expected intraclass correlations (ρ): a strictly environmental hypothesis would predict zero correlations among all separately reared pairs and a positive correlation between those reared together corresponding to that found in intact families, for example, usually about 0.5 for IQ¹; a strictly genetic hypothesis of polygenic transmission, by contrast, would predict correlations corresponding to the average proportion of shared genes, that is, 0.5 for full siblings, 0.25 for half-siblings, and 0.0 for unrelated individuals. These two hypotheses are extremes, and intermediate results would indicate the presence of both environmental and genetic influences.

The most fundamental assumption of an adoption study is that genetic and familial environmental factors are independent of each other in adoptees. We have no evidence to the contrary in the present study. The genetically related adoptee sibling pairs (FA, MA and PA) were reared in familial environments which, as measured by paternal income and social class, were uncorrelated (see Table 1); correspondingly, the absence of a correlation in social class between the biological fathers of the unrelated pairs (UT) fails to suggest that the pair members are genetically correlated.

The intraclass correlations ($\hat{\rho}$) for height, BPP and EL for each of the five groups, and for the two half-sibling groups combined, are shown in Table 2. We included height on the expectation that, whatever relationships emerged for BPP and EL, this variable would show a clear pattern of heritability. Although some genetic influence is evident, it appears anomalous that the correlation for the MA group should lie, albeit non-significantly, above that for the FA group while that for the PA group should be effectively zero. This may probably be attributed to the small group sizes and necessitates caution in interpreting our other findings.

Table 1 Means and intraclass correlations ($\hat{\rho}$) for adoptive and biological fathers' income and social class

	Annual income ($\times 1,000$ kroner)					Social class				
	No. of pairs	Mean	MS_b	MS_w	$\hat{\rho}$	No. of pairs	Mean	MS_b	MS_w	$\hat{\rho}$
Adoptive fathers										
Full sibs apart (FA)	25	5.587	7.611	11.014	-0.22	27	2.37	2.523	2.704	-0.07
Maternal half-sibs apart (MA)	32	4.524	6.397	5.377	0.05	32	2.41	2.788	1.781	0.19
Paternal half-sibs apart (PA)	26	5.073	7.361	7.980	-0.08	29	2.62	2.523	3.690	-0.22
Half-siblings apart (MA and PA)	58	4.770	6.860	6.544	0.01	61	2.51	2.642	2.689	-0.03
Unrelated reared together (UT)	22	7.202				24	4.21			
Biological fathers										
Unrelated reared together						15	1.33	1.405	1.933	-0.23

The intraclass correlation coefficient (ρ) is estimated as $\hat{\rho} = (MS_b - cMS_w) / (MS_b + cMS_w)$ where $c = d.f._w / (d.f._w - 2)$. $d.f._w = N$ pairs. Being an estimate of a ratio of variances, a negative intraclass correlation is not meaningful. The negative values in the table are those obtained from the unbiased estimator and the best estimate in the range would be $\hat{\rho} = 0$. Details of paternal income and social class were obtained from the adoption records. Income was not recorded for the biological fathers. Social class is based on a rating scale of occupation in which 0 = low and 7 = high⁴. Both income and social class are missing in some cases and the values given are derived from all available complete pairs. The mean values are for all included fathers, that is, twice the number of pairs, except for the adoptive fathers for the UT group for whom the two potentially different values, one relating to each adoption, have been averaged. Analysis of variance (ANOVA) of the adoptive fathers' means for the FA, MA, PA and UT groups yielded $F(3,184) = 4.00$, $P < 0.009$ for income, and $F(3,196) = 8.38$, $P < 0.001$ for social class. Multiple range tests (Newman-Keuls, $\alpha = 0.05$) show, for income, $UT > MA$ and $UT > PA$, and, for social class, $UT > FA$, $UT > MA$, and $UT > PA$. Note also that we have reported elsewhere¹³, for a different but comparable sample from the adoption register, that the paternal social class of adoptees, while being on average above that of the general population, does not display a smaller variance, that is, adoptees in general were raised in a representative range of social class environments.

The correlations for the BPP form a pattern conforming closely to the proposed genetic hypothesis. There are no reported studies of separately-reared adult full or half-siblings with which these results may be compared, but the only previous study of genetically unrelated adults reared together has also reported a negligible correlation (-0.03)⁸.

Studies of adoptive children have usually shown a lesser degree of genetic influence, and a correspondingly stronger familial environmental influence, on intelligence¹; perhaps both vary with age. In particular, one might expect that not all genetic effects would yet be manifested by the still-developing brain of a child⁹.

The correlations for educational level in the FA, MA and PA groups suggest a lesser degree of genetic influence on this variable and, conversely, the correlation for the UT group demonstrates a marked familial environmental effect. The latter correlation may reflect influences on scholastic aspirations and diligence. The high correlation for the FT group could thus be a result of the additive effect of both genetic and familial environmental influences.

Although the intraclass correlation remains a familiar statistic, it yields less information than a direct consideration of the within- and between-group mean squares, MS_w and MS_b respectively, from which it is derived. We have therefore also fitted biometrical genetic models to MS_w and MS_b , using maximum

likelihood^{11,14}. In a restricted model, the total variance consists of genetic variance, G , and environmental variance, E . For each sibling pair type, total variation (σ^2) is partitioned into 'within' and 'between' components: $\sigma^2 = \sigma_w^2 + \sigma_b^2$. The expectations for these components may be derived in terms of additive genetic variance, D_R , within-family environment, E_1 , and between-family environment, E_2 (ref. 11). Expected values for the observed mean squares may be readily obtained as $E(MS_w) = \sigma_w^2$ and $E(MS_b) = \sigma_w^2 + 2\sigma_b^2$ for sibships of size = 2. Table 3 gives the expected values. Assumptions in the model are: (1) no genotype-environment interaction, (2) no genotype-environment covariance, (3) $G = 1/2 D_R$, and (4) E_1 and E_2 values are the same for all types of sibship. We have no direct test of assumption (1) in these sibships. Assumptions (2)–(4) are jointly tested by the homogeneity of the total variances across sibling types (height $F_{\max}(4, 56) = 1.38$, not significant (NS); BPP $F_{\max}(5, 64) = 1.59$, NS; EL $F_{\max}(5, 64) = 1.64$, NS), and also by the goodness of fit of the models ($P > 0.34$). Assumption (2) can also be tested by the equality of the total variance of biological siblings reared together and the pooled variance of the siblings reared apart (BPP $F_{\max}(2, 161) = 1.16$, NS; EL $F_{\max}(2, 161) = 1.10$, NS).

For all three variables the model provides an adequate fit with additive genetic variance significant in all cases (see Table 4). Between-family environmental variance is significant for EL

Table 2 Means and intraclass correlations ($\hat{\rho}$) for siblings' height, intelligence (BPP) and educational level (EL)

	No. of pairs	Height (cm)				BPP				EL			
		Mean	MS_b	MS_w	$\hat{\rho}$	Mean	MS_b	MS_w	$\hat{\rho}$	Mean	MS_b	MS_w	$\hat{\rho}$
Full sibs apart	28	174.7	43.87	23.66	0.27	33.38	257.50	85.16	0.47‡	3.91	7.335	3.089	0.38*
Maternal half-sibs apart	34	175.3	63.67	28.12	0.36*	32.47	122.57	91.71	0.11	3.65	2.380	3.912	-0.27
Paternal half-sibs apart	30	175.7	31.23	34.67	-0.09	35.17	154.53	77.97	0.30*	4.13	4.446	2.667	0.22
Half-sibs apart	64	175.5	48.09	31.13	0.20*	33.73	139.02	85.27	0.22*	3.88	3.413	3.328	0.00
Unrelated reared together	24	177.0	36.69	31.42	0.03	37.75	157.22	138.88	0.02	4.96	6.214	2.292	0.43†
Full sibs reared together	73	187.5	11.24	7.25		40.59	228.87	70.45	0.52§	4.95	7.225	1.404	0.67§

Being an estimate of a ratio of variances, a negative intraclass correlation is not meaningful. The negative values in the table are those obtained from the unbiased estimator (see Table 1), and the best estimate in the range would be $\hat{\rho} = 0$. The PA and MA & PA values for height are based on 29 and 63 pairs, respectively. Height was not recorded for one of the adoptees in the PA group; $\hat{\rho}$ is not calculated for the FT group because of the range restriction—all individuals were ≥ 184 cm. ANOVA for the four adoptee groups (FA, MA, PA and UT) yielded $F(3,228) = 4.32$, $P < 0.006$ for EL, and a multiple range test (Newman-Keuls, $\alpha = 0.05$) shows $UT > FA$, $UT > MA$ and $UT > PA$. For BPP the overall comparison is not significant, $F(3,228) = 2.22$, $P > 0.09$, but an *a priori* comparison shows that the UT group is higher than the FA, MA and PA groups combined: $t(228) = 2.18$, $P < 0.03$.

* $P < 0.05$; † $P < 0.01$; ‡ $P < 0.005$; § $P < 0.001$.

Table 3 Expected mean squares for biometrical genetic model

		D_R	E_1	E_2
Full sibs apart	MS_w	1/4	1	1
	MS_b	3/4	1	1
Maternal half-sibs apart	MS_w	3/8	1	1
	MS_b	5/8	1	1
Paternal half-sibs apart	MS_w	3/8	1	1
	MS_b	5/8	1	1
Unrelated reared together	MS_w	1/2	1	0
	MS_b	1/2	1	2
Full sibs reared together	MS_w	1/4	1	0
	MS_b	3/4	1	2

D_R , Additive genetic variance; E_1 , within-family environmental variance; E_2 , between-family environmental variance.

Table 4 Biometrical genetic models

Parameter	Estimate $\pm$ s.e.	z	d.f.	Model fit χ^2	P
Height model					
D_R	53.349 $\pm$ 21.73	2.45*			
E_1	7.321 $\pm$ 12.49	0.59			
E_2	3.259 $\pm$ 7.62	0.43	5	5.2857	0.3820
BPP model					
D_R	256.20 $\pm$ 66.23	3.87†			
E_1	3.95 $\pm$ 19.97	0.20			
E_2	6.15 $\pm$ 15.99	0.38	7	4.6248	0.7056
EL model					
D_R	4.162 $\pm$ 1.818	2.29*			
E_1	0.324 $\pm$ 0.537	0.60			
E_2	1.655 $\pm$ 0.478	3.46†	7	7.8416	0.3468

* $P < 0.05$; † $P < 0.001$.

only. Biometrical genetic modelling allows estimates of the effects of dominance deviation and assortative mating^{10,11,14}; the results of the goodness-of-fit tests imply non-significance for both parameters in these data.

Some degree of familial environmental effect on intelligence and educational level can be detected in the means of the four adoptee groups. The adoptees in the UT group were reared in homes that were significantly wealthier and of a higher social class than those in the other three adoptee groups (see Table 1). Parents adopting more than one child were better situated than adoptive parents in general; this has elevated the intelligence and educational level of their adoptive children. Compared with the FA, MA and PA groups, the UT group adoptees have a significantly higher BPP and EL, the latter effect being more marked than the former (see Table 2). The mean differences in EL and BPP between the adoptees taken together (FA, MA, PA and UT) and the non-adopted siblings (FT) are not easily interpreted as either or both groups may deviate from the general population, and genetic or environmental differences may be involved.

The correlation between BPP and EL is 0.65 for all adoptees and 0.71 for the non-adopted siblings; this replicates the strong relationship usually found between intelligence and educational level¹². These two variables are generally thought to interact with each other. As our findings suggest stronger genetic effects on intelligence than on educational level, it could be that genetic influences on educational level are indirect and operate primarily through genetic effects on intelligence. Conversely, however, the effects of familial environment on educational level appear stronger than the effects on intelligence. Therefore, the effect of familial environment on intelligence might be the indirect result of a direct influence of education itself on intelligence. In view of the comparatively small sample sizes used in the present study, and the (perhaps consequent) paradoxical findings for height, this interpretation must only be tentative.

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Vertical disparity pooling and the induced effect

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If the image received by one eye is vertically magnified by a small amount then an illusory tilt is perceived around the vertical axis through the fixation point. This is known as the induced effect¹ and it can be explained by a recent computational theory of binocular vision^{2–4} which treats it as a side-effect of the use of vertical disparities to recover information about the distance to the fixation point and the angle of gaze. We have investigated the consequences of introducing vertical magnifications of some parts of a scene and not others and report here that there is a simple linear relationship between the size of the induced effect and the average vertical magnification. This suggests that a pooling strategy is adopted in the measurement of vertical disparities, a result which fits in well with expectations of the theory.

The computational theory of binocular vision^{2–4}, which demonstrates how vertical disparities can be used to discover the distance to the fixation point (d) and the angle of gaze (g) with which the fixation point is being viewed, is introduced in ref. 5. One prediction of the theory is that vertical disparities produced by a monocular vertical magnification will lead the system to compute an incorrect angle of gaze³. Since this inappropriate value of g is then used to scale the horizontal disparities an erroneous left/right tilt of the entire field of view will result. This explanation of the induced effect will be called the ' d/g hypothesis'.

We have investigated how vertical disparity information is integrated from various parts of the field of view. We wished to know whether a mixture of vertical disparities that was incoherent, in the sense that it would not arise in normal viewing of natural scenes, would destroy the induced effect because it would then be difficult to compute the correct d and g from the conflicting information³. Alternatively, since the vertical disparities observed in normal viewing are quite small and perhaps difficult to measure accurately, the visual system might adopt a pooling strategy that would be reflected in an orderly relationship between the average magnification and the size of the induced effect created.

The experimental stimulus was an array of dots seen by binocular fusion of a pair of computer-controlled high-resolution x-y displays (Hewlett-Packard 1332A) that formed

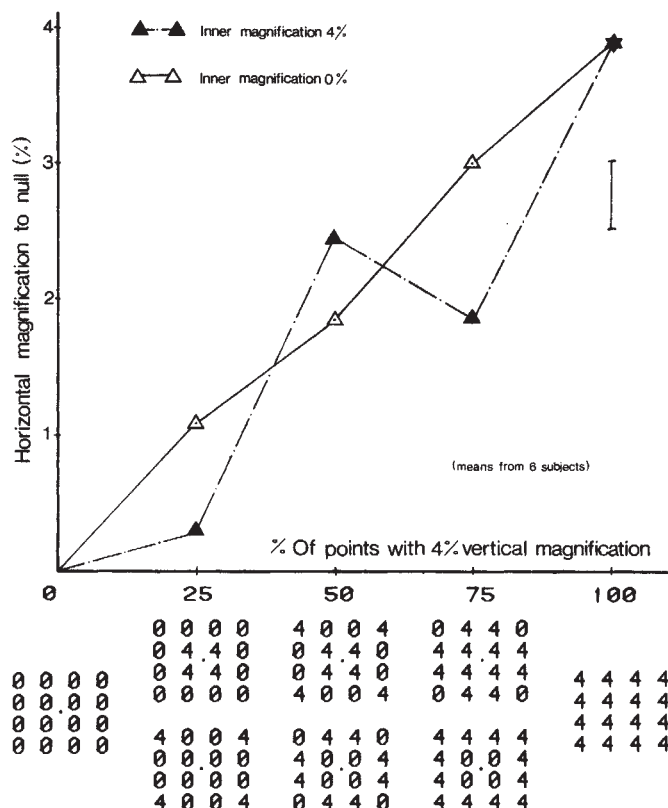


Fig. 1 Relationship between the size of the induced effect and the percentage of dots adjusted in position by a 4% monocular vertical magnification. Diagrams below the abscissa show the spatial distribution of dots whose positions were adjusted by 0% and 4% vertical magnifications. For the purposes of this graph (but not for the statistical analyses) each subject's mean setting for the 0% condition, which measured his subjective fronto-parallel plane, was subtracted from all points so that the plots illustrate directly the size of the induced effects. Solid triangles show means for the conditions where the four innermost dot positions were magnified (top row of diagrams); open triangles show means for conditions where the four innermost dot positions were unaltered (bottom row). The vertical bar on the right of the figure shows the mean standard error.

Methods: The dots had diameters of ~ 2.6 arc min, luminance of ~ 13.1 cd m^{-2} and could be positioned to an accuracy of ± 0.2 arc min. They were arranged in a regular lattice except that the position of each dot was randomly displaced by up to ± 8.5 arc min in both the x and y axis to avoid easily detectable shape cues associated with the various stimulus conditions. The overall size of the array was 7.2×7.2 deg visual angle.

the left and right fields of a Wheatstone stereoscope. Induced effects were created by altering the positions of selected dots in the right field. This simulates the effect of a meridional size lens, and gives a monocular magnification of the vertical projections of dots from the horizontal meridian through the fixation point¹. Measurement of the induced effect was by a nulling technique which cancelled the induced effect with an opposite tilt illusion called the 'geometric effect'. The latter is also an illusory tilt around the vertical axis, but in the opposite direction, and is produced when the image received by only one eye is horizontally expanded by a small amount. This illusion was produced by simulating the effect of a meridional size lens with its axis vertical.

The subject was seated in a completely dark room, with his head position fixed by a forehead and chin rest. He saw about 40 cm away from him at eye level a 4×4 array of small bright dots with a fixation point at its centre (see Fig. 1 for details). At the start of each trial the display possessed some randomly-sized left/right tilt by virtue of the induced effect being measured having superimposed upon it an initial geometric effect, ran-

domly selected within the range $\pm 4.5\%$. Subjects were not informed of the source of the tilt they saw at any one time, whether it was induced effect, geometric effect or a mixture of the two. Their task was to press a pair of buttons until the array of dots appeared to lie in a fronto-parallel plane through the fixation point. A press on one button increased the nulling horizontal magnification by 0.5%, the other decreased it by 0.5%. Subjects were allowed to track to and fro over their null region before deciding on a setting, but training sessions ensured that they were able to make each setting within ~ 15 s.

Six subjects were used, each providing 10 settings per condition but with the largest and smallest eliminated to reduce experimental noise. Each block of 10 settings required about 3–5 min and to reduce fatigue successive blocks were separated by breaks of ~ 5 min during which subjects left the experimental room. A fuller account of the experimental design will be presented elsewhere⁶.

All eight experimental stimuli are shown schematically below the abscissa in Fig. 1, where 0s and 4s indicate dots whose positions were adjusted by 0% and 4% magnification respectively. These 'mixed magnification' stimuli comprised two groups, distinguished by whether the vertical projections of the inner four dots of the array were 4% magnified or left unaltered. The two types permitted a preliminary exploration of the importance of the distribution of magnified points.

The results shown in Fig. 1 indicate that the induced effect created with uniform 4% vertical magnification was nulled by an opposing horizontal magnification of almost exactly equal size. This result replicates a well attested result regarding the relationship between induced and geometric effects¹ and suggests that the novel technique used here to measure the induced effect was satisfactory. The two types of mixed magnification stimuli did not produce significantly different tilts ($F[1, 5] = 1.258$, not significant), nor was there a significant interaction between that factor and the percentage of magnified points ($F[2, 10] = 3.036$, not significant). On the other hand, the magnitude of the induced effect increased with the percentage of magnified points ($F[4, 20] = 13.63$, $P < 0.0001$), with a trend analysis incorporating data pooled over the two types of mixed magnification stimuli showing only the linear trend to be significant ($F[1, 20] = 91.916$, $P < 0.0001$); slope of linear regression = 0.924).

This simple linear relationship suggests that the size of the induced effect is determined by the 'average vertical magnification' and implies that vertical disparity information from different parts of the field of view is pooled. The similarity between the two types of mixed magnification stimuli indicates that there is no significant difference in weighting between relatively central regions and peripheral ones. Pooling fits in well with the d/g hypothesis because viewing system parameters are intrinsically whole-field in character: as only one d and one g can exist at any one time, it makes sense to pool information about them, particularly if that information is delivered by vertical disparities which are inevitably small. The fact that vertical disparities are scaled by retinal eccentricity but unaffected by local variations in depth³ would be compatible with a pooling mechanism that allows data to be pooled over relatively large areas.

There are a variety of ways in which vertical disparity information might be pooled. These might be divided into two broad classes distinguished by whether locally computed values of d and g are averaged or whether vertical disparity information is pooled before the computation of d and g . Whatever the particular scheme adopted by the human visual system, the result reported here suggests that an orderly pooling of vertical disparity information does occur.

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Antibodies to heavy neurofilament subunit detect a subpopulation of damaged ganglion cells in retina

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Neurofilaments (NFs) consist of three protein subunits with apparent molecular weights of 68,000 (68K), 145K and 200K, which are found closely associated in most but not all locations in the nervous system¹⁻⁴. One of these exceptions is the inner retina of the mouse, where antibodies to 145K NFs label large ganglion cells throughout the extent of the cells, while antibodies to 200K NFs label only more distal portions of the optic axons but usually fail to label the ganglion cell somata and proximal axons^{5,6}. Very rarely, however, and more often in old mice, anti-200K NF antibodies do label a ganglion cell completely⁵. To determine whether these rare, completely labelled cells reflect a pathological alteration, we cut the optic axons, and report here that after a few days some of the axotomized cells could be labelled completely, in a Golgi-like fashion, by anti-200K NF antibodies. These cells seem to represent the population that forms the projection to the bulk of the lateral geniculate nucleus, as suggested by their size, distribution and projection pattern. Hence, antibodies to the heavy NF subunit in combination with lesions may allow selective retrograde tracing of a subpopulation of ganglion cells, and such antibodies can be used to detect damage in NF-rich neurones at a very early stage, long before they eventually degenerate.

Neurones respond to axonal injury with the retrograde cell reaction—chromatolysis and displacement of the nucleus—which may be followed by recovery, or, for centrally projecting neurones in mammals, often by degeneration of the entire cell^{7,8}. Lesions of optic axons cause retrograde degeneration of retinal ganglion cells in mammals, which in adults is a rather slow process that may take months to years^{9,10}. The change in affinity to NF antibodies in response to axotomy described here for the retina represents another feature of the retrograde reaction that is pronounced only in the largest ganglion cells. A similar abnormal neurofilament labelling has been observed in lesioned peripherally projecting neurones, where it was attributed to neurofibrillar hypertrophy¹¹. The abnormality in the retina, however, reflects primarily an antigenic change rather than an increase in neurofilaments.

The binocular visual field in the mouse is largely located above the head, and is seen by the lower temporal retina. Some of the ganglion cells in this retinal region project to the brain on the same side, where they join the projection from the opposite eye, a convergence that forms the substrate for binocular vision¹². Thus, when the optic tract on one side is lesioned, the effect of axotomy can be compared with the normal condition in the same retina, ipsilateral to the lesion. In the nasal retina, representing normal conditions (Fig. 1b), optic axons labelled by anti-200K NF antibodies can be followed from the optic disk for only a limited distance; ganglion cell somata and proximal stretches of axons remain invisible. In contrast, in the lesioned cells in the lower temporal retina (Fig. 1a) the reaction with the antibodies extends to the proximal axons, cell somata and dendrites, as also seen at higher power in Fig. 2. The labelled cell bodies were large ($17.2 \pm 1.8 \mu\text{m}$ mean diameter, $n = 207$), rather regularly spaced, and were probably identical to the large, NF-rich ganglion cells seen in normal mouse retina using methods that detect NF subunits of lower molecular weight^{6,13,14}. Camera lucida drawings of all labelled ganglion cells ipsilateral to the optic tract lesion (Fig. 3a) showed about 300 cells (312 ± 14 , $n = 6$), representing about one-third of all ipsilaterally projecting cells in the mouse¹².

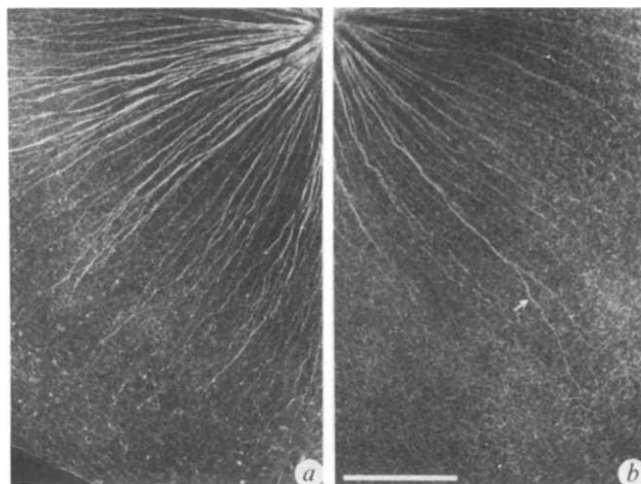


Fig. 1 Whole-mount of mouse retina showing effect of axotomy. A young adult C57BL/6J mouse was anaesthetized with Nembutal, and one optic tract was exposed and sectioned. After 10 days the mouse was perfused with paraformaldehyde and the retinas were reacted with an anti-200K NF antibody; here RT97 was applied²¹, which gave results similar to the anti-200K NF antibodies R3 (ref. 5), 06-17 (ref. 22), 4.3F9 and 2.2F3 (ref. 23) used in other preparations. Antibody binding was visualized with a fluorescein isothiocyanate-labelled secondary antibody (Boehringer-Mannheim). Shown here are sectors from the retina ipsilateral to the optic tract lesion. *a* Includes part of the retinal representation of the binocular visual field which sends a projection to the ipsilateral side of the brain. *b*, Retinal sector corresponding to the monocular field represented only in the contralateral brain; hence the ganglion cells here were not affected by the optic tract lesion. The position of the optic disk, just above the upper margin in both views, can be inferred from the converging optic axons; the ciliary edge of the retina is visible in the lower corners of the figure. The textured background of the entire retina is due to labelling of the NF-rich axonless horizontal cells in the outer retina²⁴. Note that in the unaffected retinal sector (*b*) the labelling of optic axons increases in intensity towards the optic disk, and even the longest axon segments visible cannot be traced much further than about half-way through the extent of the retina. In the sector containing axotomized ganglion cells (*a*), however, some optic axons can be followed all the way to their cell bodies, which are barely visible at this magnification in the peripheral retina. The increasing affinity of anti-200K NF antibodies for more distal portions of axons in normal conditions is further illustrated by the fibre indicated by an arrow in *b*: for this fibre the segment in the retina was the distal portion of the axon; it entered the retina through the optic disk and gave off branches on its course, a characteristic of efferent fibres (called efferent with respect to the brain²⁵). It is unknown where the cell bodies of such efferent fibres in the mouse are located. Scale bar 500 μm .

In contrast to the limited retinal region giving rise to the ipsilateral retinofugal projection, contralaterally projecting ganglion cells are known to originate from the entire retina, and to outnumber the ipsilateral projection by about 60 to 1 in the mouse¹². After the induction of optic tract lesions, however, the ganglion cells that assumed Golgi-like affinity to 200 K NF antibodies in the retina contralateral to the lesion formed a small subpopulation: about 1,000 cells were labelled, representing ~2% of the contralateral projection. The labelled cells were large and regularly distributed throughout the retina except for the lower temporal region which consistently contained only a scattered few labelled cells; this sparsely labelled region corresponded to the ipsilaterally projecting region in the other retina (Fig. 3b). Hence, a lesion of optic axons caused abnormal affinity for anti-200K NF antibodies in a subpopulation of ganglion cells which cover the retina more or less evenly and which have a line of decussation in their projections to one or the other side of the brain.

Such a pattern of projections is compatible with retinal inputs to the geniculo-cortical system but not to the optic tectum, as

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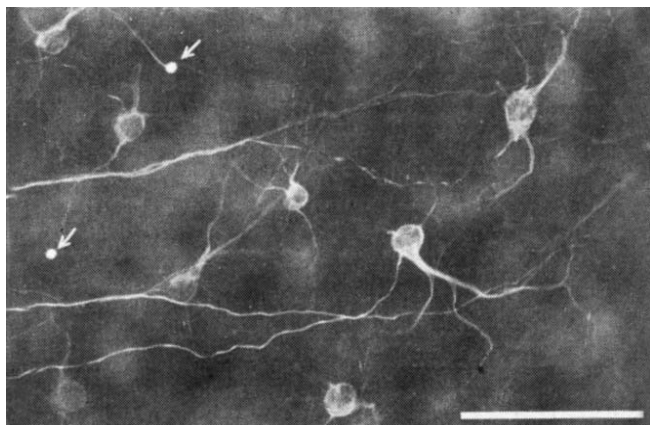


Fig. 2 Ganglion cells in retina ipsilateral to an optic tract lesion made 15 days previously, labelled for 200K neurofilaments with RT 97 (ref. 21). These cells were the largest ganglion cells and formed about one-third of the entire ipsilateral projection¹². Arrows indicate neurofilamentous clubs⁸ that may represent the axons of some of the smaller axotomized ganglion cells. Such neurofilamentous clubs appeared a few days after the optic tract lesion and increased in frequency with time. They are also visible as bright specks in Fig. 1a. Scale bar, 100 μ m.

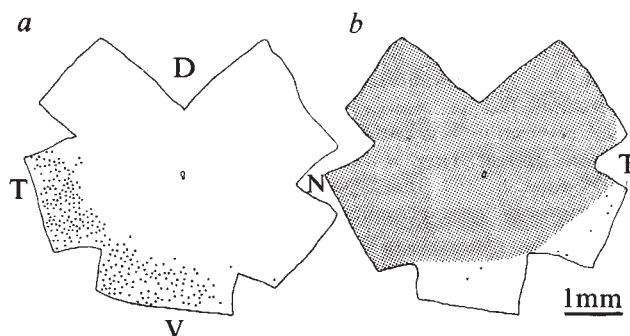
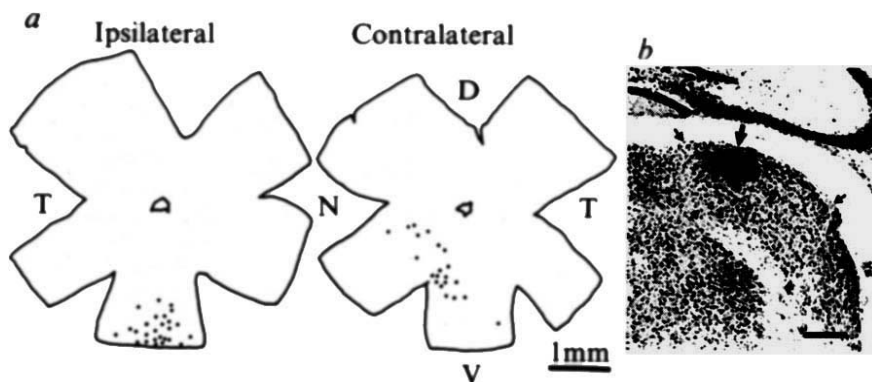


Fig. 3 Camera lucida tracings of ganglion cells labelled by anti-200K NF antibodies after optic tract section 6 days previously. Ipsilateral to the lesion (a) the labelled ganglion cells were regularly spaced within a crescent-shaped area in the ventral and temporal retina. The inner border of this crescent corresponds to the zero vertical meridian of the visual field¹². Contralateral to the lesion (b) the bulk of the labelled cells were located in the shaded area. Their number could only be estimated ($\sim 1,000$) because in more central retinal regions they were frequently obscured by heavily labelled bundles of optic axons. A crescent-shaped area corresponding to the ipsilateral projection was largely free of labelled ganglion cell bodies except for a scattered few. This region did, however, contain neurofilamentous clubs like those described in Fig. 2. V, ventral; D, dorsal; N, nasal; T, temporal.

Fig. 4 Identification of ganglion cells projecting to the geniculate, using horseradish peroxidase (HRP) injections. Mice were anaesthetized with Nembutal, and visual responses were recorded from the lateral geniculate nucleus, using standard electrophysiological techniques with KCl-filled glass pipettes²⁶. When a desired location within the geniculate was identified, the electrolyte was exchanged *in situ* against a solution of HRP, a tiny amount of which was then ejected electrophoretically. After 2 days the mice were perfused and the brains and retinas reacted for HRP. **a**, Camera lucida tracings of retinal whole-mounts showing positions of ganglion cells back-filled with HRP from a location in the lateral geniculate nucleus representing the upper binocular field of vision.



b, Coronal section through the geniculate, reacted for HRP and counterstained with cresyl violet; the injection site is indicated by the large arrow, the geniculate borders by small arrows; scale bar, 200 μ m. The labelled cells were from the largest population: 17.9 μ m mean diameter ($n = 53$) ipsilateral to the injection and 15.6 μ m ($n = 176$) contralaterally, and they were regularly spaced with comparable density and cell numbers in the two retinas. All injections into the main body of the lateral geniculate resulted in similar retinal labelling patterns, but it is possible that a population of smaller ganglion cells project to the outer shell of this nucleus. Comparable small injections into the most superficial layer of the superior colliculus label tight clusters of small ganglion cells, and deeper regions of the visual collicular layers receive a sparse and divergent input from large ganglion cells, which may be a collateral projection from the same cells that supply the lateral geniculate nucleus²⁶.

judged from physiological recordings. Each tectum contains a map of the entire contralateral retina, and the ipsilateral eye influence is very sparse^{15,16}. The visual field representation in cortex, however, does not include the ipsilateral field (except for some infringement at the midline), and within the binocular field the influence of the ipsilateral eye is comparable with that of the contralateral eye¹⁷. However, although physiological observations point to a line of decussation in retinogeniculate projections, such a dividing line has never been observed anatomically in adult rodents (ref. 18 but see ref. 19). To identify the ganglion cells projecting to the geniculate, we injected horseradish peroxidase (HRP) into this nucleus. All injections confined to the main part of the geniculate labelled a population of largest ganglion cells that were spaced widely and rather regularly. When HRP was injected into the representation of the binocular field, populations of ganglion cells similar in size and spacing were labelled in both eyes (Fig. 4). In spacing, cell size and comparable bilateral distributions, the projections to the geniculate were indistinguishable from the cells that expressed the NF abnormality following optic tract lesions. This makes it likely that the line of decussation in the projections of the NF-rich ganglion cells is the anatomical substrate that

accounts for the physiologically observed absence of ipsilateral field representation in visual cortex.

The time lapse to the appearance in the cell bodies of the antigenic change in response to lesions depended on the distance of the axotomy from the eye; it took about 6 days following optic tract lesions, and about 3 days after optic nerve cuts, suggesting the retrograde transmission of an injury message at ~ 1.5 mm per day. The antigenic change persisted for several weeks, but seemed to reverse gradually; 60 days after the lesions only a few cell bodies were still labelled, but the labelled axon segments were still extended, indicating that the cells had probably not degenerated yet but were undergoing some temporary recovery.

Here we have shown that antibodies to the heavy NF subunit which normally fail to label proximal neuronal regions, do so following lesions of the axons. The inability to label proximal cells normally may reflect the absence of the heavy NF subunit from this location, or, more likely, an affinity of the antibodies for a modified site that is slowly created as the neurofilaments are transported down the axon. The latter explanation was suggested by the Sternbergers, who showed that several anti-200K NF antibodies are directed against phosphorylated sites

not present in cell bodies²⁰. Thus, one possible explanation is that the pathological changes observed here indicate atopic phosphorylation of neurofilaments in response to injury.

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Amplification of specific DNA sequences correlates with multi-drug resistance in Chinese hamster cells

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Mammalian cells selected for resistance to certain cytotoxic drugs frequently develop cross-resistance to a broad spectrum of other drugs unrelated in structure to the original selective agent^{1,2}. This phenomenon constitutes a major problem in cancer chemotherapy. Multi-drug resistance arises from decreased intracellular drug accumulation^{1–4}, apparently due to an alteration of the plasma membrane^{2,5–7}. The observation of double minute chromosomes or homogeneously staining regions in some of the multi-drug-resistant cell lines^{8–12} suggests that gene amplification underlies this phenomenon. We have used the technique of DNA renaturation in agarose gels¹³ to detect, compare and clone amplified DNA sequences in Adriamycin- and colchicine-resistant sublines of Chinese hamster cells^{3,12}. We show that both Adriamycin- and colchicine-resistant cells contain amplified DNA fragments, some of which are amplified in both of these independently derived cell lines. Furthermore, loss of the multi-drug resistance phenotype on growth in the absence of drugs correlates with the loss of amplified DNA. These results strongly suggest that the DNA sequences which are amplified in common in multi-drug-resistant cell lines include the gene(s) responsible for a common mechanism of multi-drug resistance in these cells. We have cloned one of the commonly amplified DNA fragments and show that the degree of amplification of this fragment in the cells correlates with the degree of their drug resistance.

Table 1 Multi-drug resistance and 1.1-kb fragment amplification in different cell lines

Cell line	Relative resistance to:		Degree of 1.1-kb fragment amplification
	Adriamycin	Colcemid	
V79	1	1	1
77A	5	7	5
LZ	3,000	436	60
LZ revertant	7		10
CHO Aux B1	1	1	1
C5	150	280	20

Isolation and characterization of the Adriamycin-resistant sublines of V79 cells, 77A and LZ, have been described previously^{12,23}. Cell lines CHO Aux B1 and C5 (ref. 3) were gifts from Dr V. Ling (University of Toronto). LZ revertant cells were obtained by growing LZ cells for 130 cumulative doublings in the absence of drug selection¹². The degree of drug resistance of C5 cells was determined as described²³. The degree of amplification of the 1.1-kb fragment I in different cell lines was determined from Southern blots (Fig. 2a) and from slot-blots (Fig. 2b) by cutting out and counting the bands of ³²P-labelled DNA.

The Chinese hamster cell lines used here are listed in Table 1. LZ, a highly Adriamycin-resistant subline of V79 cells, was obtained from a weakly resistant line 77A by multistep selection with increasing concentrations of Adriamycin¹³. The 3,000-fold degree of Adriamycin resistance in the LZ line is unstable, as LZ cells revert to an ~7-fold degree of Adriamycin resistance after prolonged growth in the absence of the drug¹². In addition to Adriamycin, LZ cells are cross-resistant to several other drugs, including colcemid, a close analogue of colchicine¹². C5, an independently derived subline of CHO cells selected for resistance to colchicine, is also resistant to multiple cytotoxic drugs³. LZ cells are relatively more resistant to Adriamycin than to colcemid (Table 1), whereas C5 cells are more resistant to colcemid than to Adriamycin. Indeed, it has been frequently observed that multi-drug-resistant cell lines are relatively more resistant to the drug used for their selection^{1,2,8,10}. Such specificity suggests that while a common genetic alteration may have led to multi-drug resistance in different cases, additional genetic events may have further modified the pattern of drug resistance in different lines.

DNA from different cell lines was digested with *Bam*HI, and a portion of each DNA preparation (tracer DNA) was ³²P-labelled with T4 DNA polymerase¹³. Tracer DNA was mixed with an excess of unlabelled (driver) DNA and electrophoresed in an agarose gel. Following electrophoresis, DNA in the gel was subjected to two cycles of denaturation, renaturation and *in situ* digestion with S₁ nuclease to enhance selectively the signal from reiterated DNA fragments¹³. When both tracer and driver DNA derive from the same DNA preparation, this treatment produces a series of bands corresponding to the *Bam*HI restriction fragments that are reiterated at least 15–30 times per haploid mammalian genome. When tracer and driver DNA are from different DNA preparations, only those DNA fragments that are reiterated both in the tracer and in the driver are detected¹³. As the amplified chromosomal domains are known to contain varying amounts of flanking sequences that have been co-amplified together with the essential gene^{14,15}, identification of the commonly amplified sequences in different independently derived cell lines by the above procedure allows one to delineate the region which is likely to contain the gene of interest.

Figure 1 shows the results of experiments in which DNA was electrophoresed and then subjected to the treatment described above in either a 1% (Fig. 1a–l, u–x) or 0.7% (Fig. 1m–t) agarose gel. Several bands corresponding to repeated *Bam*HI fragments of hamster DNA are observed in the digest of parental V79 DNA (Fig. 1a, m, u). DNA from the weakly resistant line 77A (Fig. 1b, n) gives a pattern which is indistinguishable from that of V79. However, in the digest of LZ DNA (Fig. 1c, o, v)

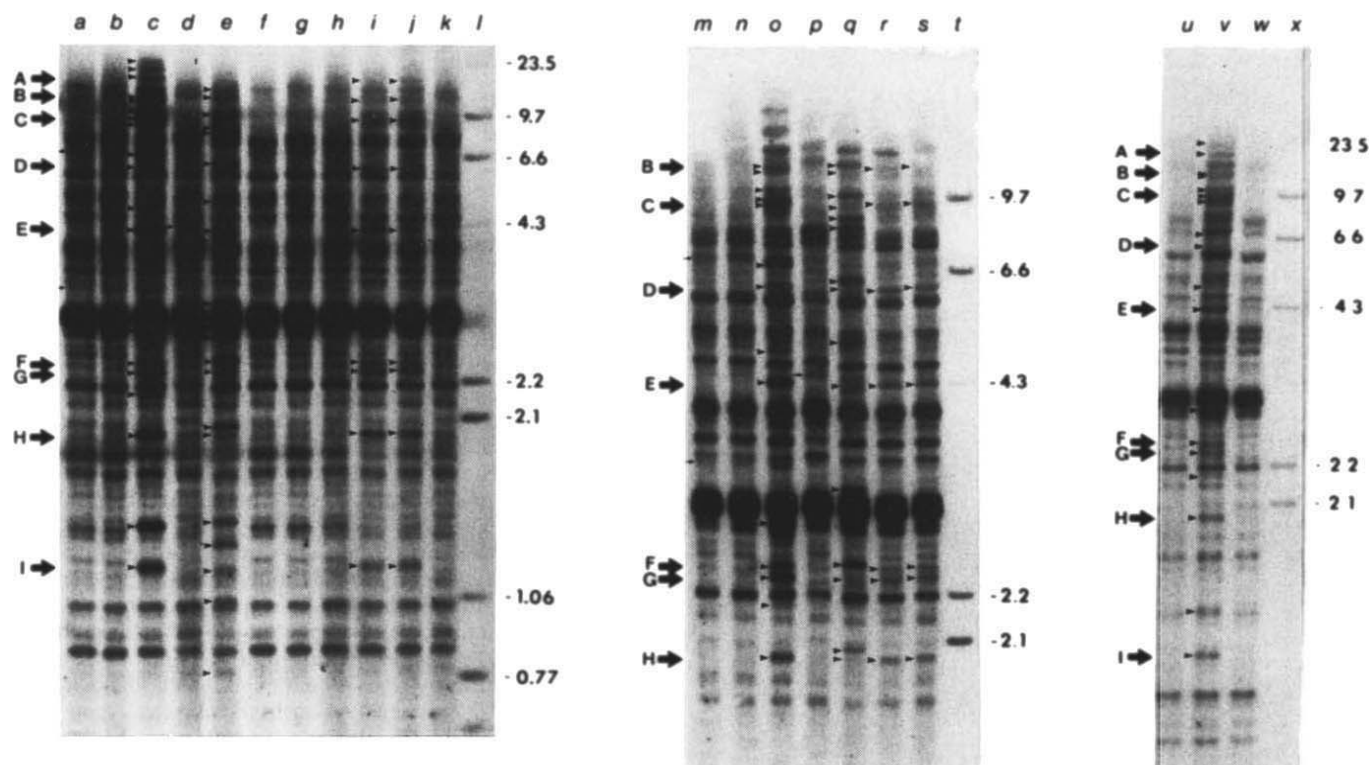


Fig. 1 Identification of amplified DNA fragments by in-gel DNA renaturation. Lanes *a-l*, *v-x*: 1% agarose gel; lanes *m-u*: 0.7% agarose. Each lane contains *Bam*HI digests of the following DNA samples: *a*, *m*, *u*, V79 (tracer and driver); *b*, *n*, 77A (tracer and driver); *c*, *o*, *v*, LZ (tracer and driver); *d*, *p*, CHO Aux BI (tracer and driver); *e*, *q*, C5 (tracer and driver); *f*, LZ (tracer), V79 (driver); *g*, *γ*, hLZ (tracer), 77A (driver); *h*, 77A (tracer), LZ (driver); *i*, *r*, LZ (tracer), C5 (driver); *j*, *s*, C5 (tracer), LZ (driver); *k*, C5 (tracer), CHO Aux BI (driver); *w*, LZ revertant (tracer and driver). *Hind*III fragments of λ phage DNA (*l*, *t*, *x*) and *Hinc*II fragments of Φ X174 RF DNA were used as size standards (*l*, *t*, *x*). Amplified DNA fragments are indicated by arrowheads. A subset of DNA fragments amplified in both LZ and C5 cells (*A-I*) are marked by arrows. Small arrows indicate the differences between the V79 and CHO DNA patterns (*a*, *d*, *m*, *p*). Several bands at the top of lanes *m-t* are missing because of gel breakage in this experiment.

Methods: DNAs from the different cell lines were extracted by the procedure of Blin and Stafford²⁴. DNA was digested with *Bam*HI and tracer DNA was labelled with ³²P using T4 DNA polymerase and [α -³²P]dCTP (3,000 Ci mmol⁻¹) in conditions where an average of ~100 nucleotides were excised from each 3' end in the exonuclease reaction before resynthesis¹³. DNA was separated in a 30.5 × 24 × 0.4 cm agarose gel. After electrophoresis, the gel slab was cut to 28.5 × 16.3 cm, placed in a flat-bottomed plastic box and subjected to two cycles of in-gel renaturation and S₁ nuclease digestion as described¹³ with the following modifications. The composition of the hybridization buffer was 0.9 M NaCl, 0.5 mM EDTA, 50% formamide, 0.05 M Na-phosphate (pH 7.0). The buffer volume used for washes was 350 ml, and S₁ nuclease digestion was done in 250 ml of digestion buffer¹³ containing 20,000 units of S₁ nuclease, after five 15-min washes with the digestion buffer. Each lane was loaded with either 1.36 × 10⁷ d.p.m. in 0.35 μ g (*a-k*, *m-t*) or 1.0 × 10⁷ d.p.m. in 0.5 μ g (*u-x*) of tracer DNA and 15 μ g of unlabelled driver DNA.

a large number of additional bands can be detected, indicating the presence of amplified DNA in these highly drug-resistant cells. These bands were undetectable when V79 or 77A DNA was used either as a tracer or as a driver in hybridization with LZ DNA (Fig. 1*f*, *g*, *h*). The sizes of the amplified DNA fragments in the LZ DNA are ~150 kilobases (kb) in total, which should be considered the minimum estimate for the size of the amplified chromosomal domain in LZ cells (assuming that all of the amplified fragments are contiguous in the genome). In contrast, the amplified fragments were not detected in the DNA of the LZ cells that have reverted to a low degree of drug resistance after prolonged growth in the absence of Adriamycin (Fig. 1*w*). The loss of the amplified DNA fragments thus occurred concurrently with the loss of drug resistance in these cells, strongly suggesting that amplification of these DNA sequences underlies the drug resistance phenotype in LZ cells.

Comparison of the in-gel renaturation patterns produced by DNA from colchicine-resistant C5 cells (Fig. 1*e*, *q*) and from parental CHO DNA (Fig. 1*d*, *p*) reveals the presence of several amplified *Bam*HI fragments in C5 DNA; these fragments were not detected when CHO DNA was used as a driver (Fig. 1*k*). The amplified fragments in LZ and C5 DNA were compared in experiments in which LZ DNA was used as a tracer and C5 DNA as a driver (Fig. 1*i*, *r*) and vice versa (Fig. 1*j*, *s*); from the results we identified nine fragments (*A-I*) of total size ~55 kb that have been amplified in both LZ and C5 genomes. Amplifica-

tion of the same DNA sequences in two cell lines that had been independently selected for resistance to different drugs strongly suggests a common mechanism for multi-drug resistance in these two cell lines. The gene(s) responsible for this mechanism is probably contained in these commonly amplified DNA sequences.

While the DNA fragments that are amplified in both LZ and C5 cells appear as a subset of strong bands in LZ DNA (Fig. 1*c*, *o*), the corresponding bands in C5 DNA (Fig. 1*e*, *q*) are barely detectable against the background. On the other hand, the digest of C5 DNA contains several strong bands of amplified DNA with a total size of ~70 kb, none of which is also amplified in LZ DNA. The result suggests that C5 DNA contains at least two different sets of amplified DNA fragments, which may correspond to two different amplified chromosomal domains. The domain that overlaps with the amplified domain in LZ DNA is amplified weakly in C5 cells, while the other set which is unique to C5 DNA is amplified to a greater degree. The latter sequences may carry the gene(s) specifying an additional mechanism for drug resistance in C5 cells, possibly specific for cells selected for colchicine resistance.

To address the nature of the gene(s) contained in amplified DNA, we have cloned one of the *Bam*HI fragments that have been commonly amplified in LZ and C5 DNA (the 1.1-kb fragment I in Fig. 1). LZ DNA (100 μ g) was digested with *Bam*HI and separated in a 1% agarose gel. Positions of the

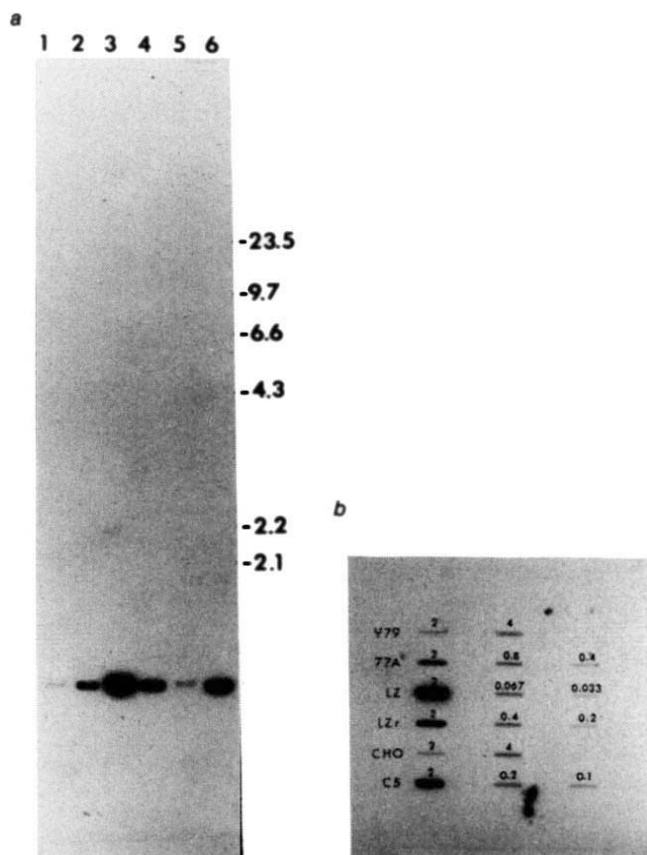


Fig. 2 Southern blot (a) and slot-blot (b) hybridization of the plasmid pDR1.1 with genomic DNA. a, DNA was extracted from V79, 77A, LZ, LZ revertant, CHO Aux B1 and C5 cells (lanes 1–6, respectively) and digested with *Bam*HI. Positions of the size standards are indicated. b, The source and amount (in μ g) of DNA in each slot are indicated.

Methods: a, Cellular DNA (5 μ g) was digested with *Bam*HI, electrophoresed in a 1% agarose gel and transferred to a nitrocellulose filter¹⁸, then hybridized with 3×10^7 d.p.m. of pDR1.1 plasmid DNA (specific activity 2×10^8 d.p.m. μ g⁻¹) that was labelled with ³²P by nick-translation²⁵. Hybridization was done at 65 °C in 5 $\times$ SSC, 2 $\times$ Denhardt's, 0.1% SDS, 100 μ g ml⁻¹ denatured salmon sperm DNA. After hybridization the filter was washed with 0.1 $\times$ SSC, 0.5% SDS at 65 °C and autoradiographed. b, DNA was bound to nitrocellulose filters as described¹⁹. The blot was hybridized with 3×10^7 d.p.m. of the pDR1.1 probe. Hybridization and washing were as described for a.

amplified fragments were established by in-gel renaturation¹³ of the lanes containing ³²P-labelled V79 and LZ DNA digests separated in the same gel. A narrow strip of the gel containing fragment I was cut out and subjected to two cycles of denaturation and renaturation. S₁ nuclease was not used in this protocol so that the reannealed fragments would retain the cohesive ends generated by *Bam*HI. The mixture of single-stranded and double-stranded DNA (the latter enriched in repeated DNA fragments after renaturation) was eluted from the gel and ligated to the *Bam*HI-cleaved pBR322 plasmid. Only double-stranded DNA could be ligated at this step. The recombinant plasmids were used to transform *Escherichia coli* HB101 (ref. 16), and plasmid DNA from the resulting ampicillin-resistant/tetracycline-sensitive colonies was analysed by mini-scale DNA extraction¹⁷ and digestion with *Bam*HI and *Hinf*I. Of 35 samples of the recombinant plasmids, seven produced identical restriction patterns, indicating that the corresponding clones contained DNA inserts that were reiterated in the genome (data not shown). DNA from one of these clones, designated pDR1.1, was used as a probe for Southern hybridization¹⁸ with *Bam*HI-digested

genomic DNA. Figure 2a shows that the cloned 1.1-kb fragment is amplified in all drug-resistant cell lines relative to the parental lines, and that pDR1.1 does not hybridize to any bands other than the 1.1-kb fragment and therefore does not contain interspersed DNA repeats. The degree of amplification of the 1.1-kb fragment was quantitated using both Southern blot¹⁸ (Fig. 2a) and slot-blot¹⁹ (Fig. 2b) hybridization procedures. The results of these assays, summarized in Table 1, indicate an approximately linear correlation between the degree of amplification of the 1.1-kb fragment and the degree of Adriamycin resistance in the weakly resistant cell lines, while in the highly resistant lines LZ and C5, the relative increase in drug resistance strongly exceeds the relative increase in the copy number of the 1.1-kb fragment. The latter result may be due either to additional genetic changes in the highly resistant cell lines (such as the amplification of the second set of DNA fragments in C5 cells discussed above), or to changes in transcriptional regulation of the amplified gene(s) at a high degree of amplification, or to the saturable nature of a biological process regulated by the product of the amplified gene(s).

The finding that gene amplification underlies the multi-drug resistance phenotype is of particular interest as this type of drug resistance does not result from overproduction of the drug targets³, which is the case in all the other known instances of drug resistance due to gene amplification^{20,21}. The nature of the protein(s) encoded by the amplified gene(s) is unknown, although the 170,000-molecular weight membrane glycoprotein and the 19,000-molecular weight cytosol protein that are frequently overproduced in multi-drug-resistant cells^{1,2,6,10,22,26,27} are likely candidates. Recently, we have isolated several phage clones containing the sequences of pDR1.1 from a genomic library of LZ DNA (P. Gros and D.E.H., unpublished). These clones are being analysed for the presence of transcribed DNA sequences and for the ability to transfer multi-drug resistance via DNA transfection. Once the specific DNA segments containing the gene(s) responsible for multi-drug resistance are identified, they will be used to isolate the corresponding protein(s). Understanding the phenomenon of multi-drug resistance in tumour cells may help in the design of treatments that would be selectively cytotoxic for the resistant cells.

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Bone marrow cells give rise to distinct cell clones within the thymus

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The thymus is the major, if not the sole site of maturation of T lymphocytes from their haematopoietic precursors¹. During embryonic life (at a few well-defined intervals, at least in birds²) the thymus receives thymus-homing haematopoietic precursors that give rise to antigen-specific functional T lymphocytes³⁻¹⁰. Although the number and thymic location of distinct T-cell lineages destined to form the peripheral T-cell pool are not yet well defined, at least two independent pathways have been proposed. First, thymic sub-capsular lymphoblasts divide and differentiate to give rise to small deep cortical thymic lymphocytes, medullary lymphocytes^{11,12} and thymus emigrants (I.W., unpublished data) and second, the medulla contains an independent self-renewing population that contains the precursors of the peripheral T-cell pool¹³. Following irradiation the thymus may be repopulated by injected haematopoietic cells presumably related to the thymus-homing haematopoietic cells of the embryo^{14,15}. Here we have reconstituted irradiated mice with limiting numbers of bone marrow cells from Thy-1 congenic donors and have found distinct clones of cells within the thymus. The pattern of reconstitution by the precursor cells indicates that two independent thymus lineages exist: cortex plus medulla, and medulla alone.

While analysing thymic repopulation in allogeneic bone marrow chimaeras we noted one chimaera¹⁶—in which the allogeneic bone marrow cells differed from the host for both major histocompatibility complex (MHC) encoded antigens and Thy-1 type—that showed one lobe of the thymus entirely reconstituted with donor type Thy-1 cells, whereas the other lobe was entirely reconstituted with host-type Thy-1 cells (Fig. 1). Wallis *et al.*¹⁷ observed that in radiation chimaeras only a few chromosomally-marked cells are needed to reconstitute an entire thymus and we were concerned that a selection process based on MHC allogenicity could have accounted for this remarkable reconstitution of two thymic lobes by two distinct stem cells. Therefore, we undertook a series of studies in which mixtures of bone marrow cells depleted of mature Thy-1⁺ cells (by extensive

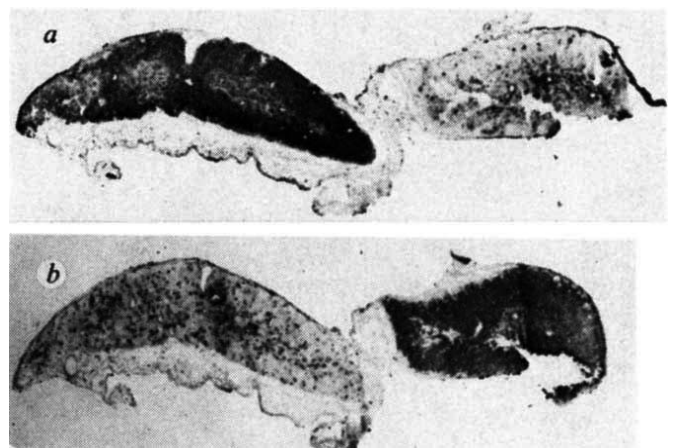


Fig. 1 Thymus from an irradiated (850 rad) C57BL/6H-2^k (Thy-1.2) mouse 1 month post-reconstitution with 5×10^6 bone marrow cells (twice treated with anti-Thy-1 + complement) from a C57BL/KaThy-1.1 (H-2^b) donor stained with *a*, anti-Thy-1.1 and *b*, anti-Thy-1.2. Immunoperoxidase stains of frozen sections²⁰ were performed with monoclonal rat anti-Thy-1.2 (clone 30-H12, Becton-Dickinson) and biotin conjugated monoclonal mouse anti-Thy-1.1 (clone 19x5) (gift of R. Nowinski) followed by rabbit anti-rat and peroxidase conjugated swine anti-rabbit (Dako) or peroxidase conjugated avidin (Vector). The left lobe is virtually entirely repopulated by donor type cells and the right lobe is virtually entirely repopulated by host type cells. Only infrequent individual cells of the opposite type are seen in each lobe.

anti-Thy-1 + complement treatment) were injected intravenously into lethally irradiated Thy-1 congenic hosts. Mice were reconstituted with bone marrow cells varying in the ratio of donor to host Thy-1 type from 1:10 to 1:100, and thymuses were taken from hosts 2–10 weeks post-irradiation. The patterns of thymocyte repopulation were examined immunohistochemically on frozen sections using monoclonal anti-Thy-1.1 and anti-Thy-1.2 antibodies.

We have examined 80 thymus lobes among which 35 contained either extremely rare or no donor cells. The following analysis is based on the 45 lobes containing significant repopulation by donor type cells. In 5 of these lobes, discrete confluent foci of the minority population in the bone marrow mixture (the donor type) were present and ranged in size from 20 to 30 cells

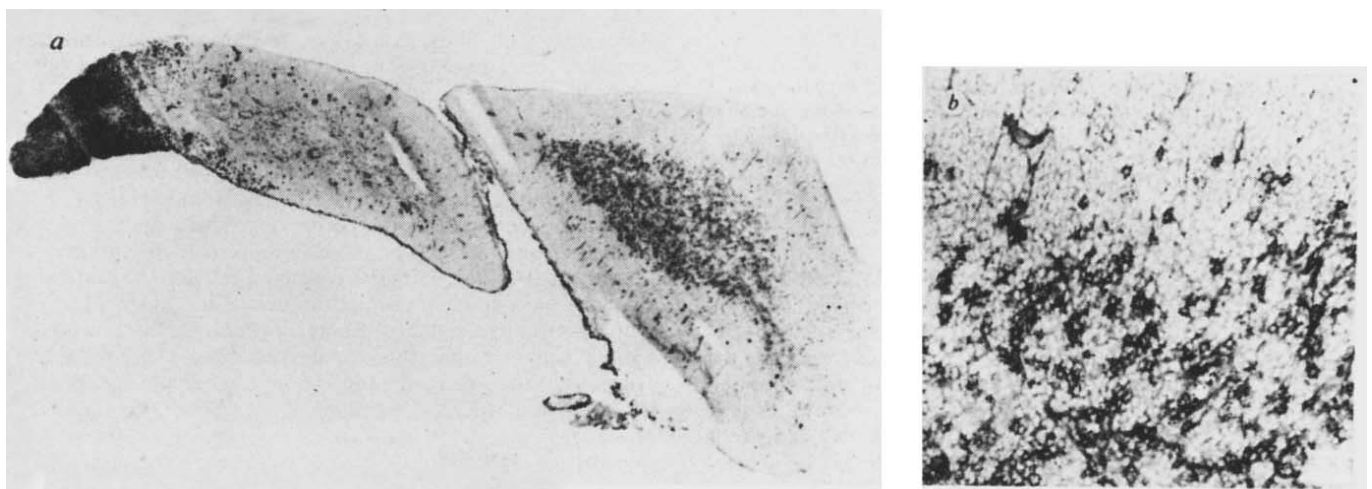


Fig. 2 Thymus of an irradiated (850 rad) C57BL/KaThy-1.1 mouse reconstituted with a mixture of anti-Thy-1 + complement treated bone marrow cells from identical and congenic (C57BL/Ka, Thy-1.2) mice, total dose 2.5×10^6 cells, Thy-1.2:Thy-1.1 (1:50), 4 weeks after reconstitution and stained here with anti-Thy-1.2 as described in Fig. 1. In this experiment and all others less than 1% of the remaining viable cells were Thy-1 positive. *a*, The left lobe contains a discrete confluent focus of donor type cells while the right lobe contains large numbers of donor type cells in the medulla with only very rare cells in the cortex. *b*, High-power view of cortex (top edge) and medulla (bottom) of right lobe, illustrating donor type (Thy-1.2) cells only in medulla.

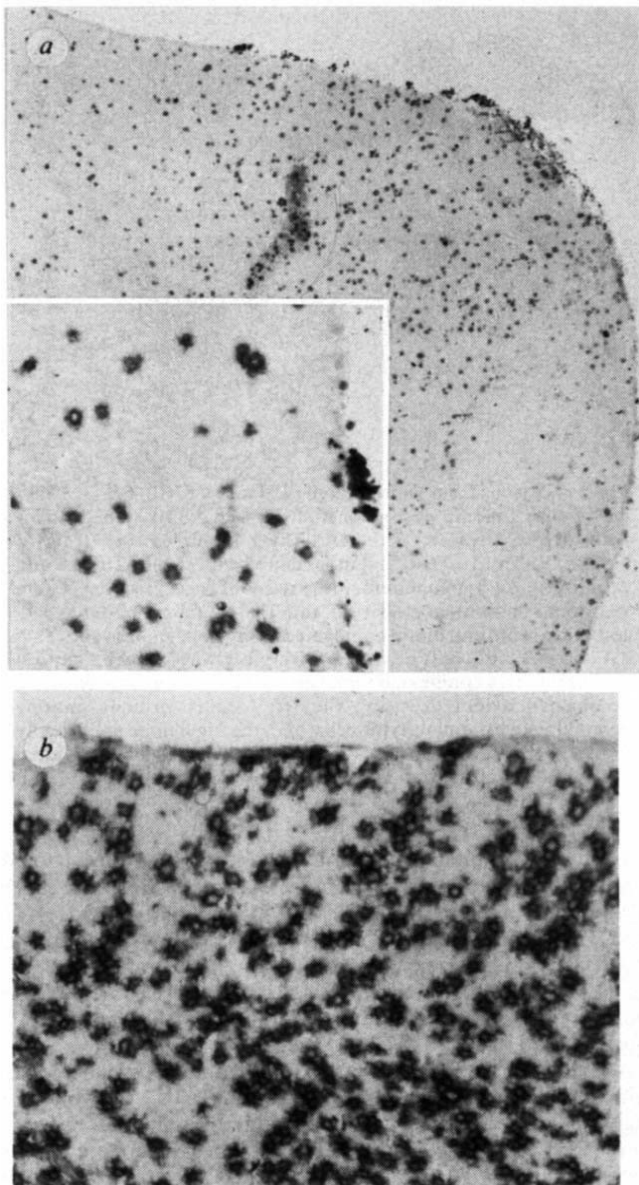


Fig. 3 Thymus of same mouse strain and reconstituted with the same total number of cells as Fig. 2, but with *a*, Thy-1.2:Thy-1.1 = 1:10, stained with anti-Thy-1.2 6 weeks after reconstitution and *b*, Thy-1.2:Thy-1.1 = 1:100, stained with anti-Thy-1.2 28 days after reconstitution. In *a* focal repopulation with scattered individual cells can be seen (cortex in lower right is negative). Inset: high-power view of individual cells. *b*, Scattered clusters of cells.

in diameter to areas of repopulation which occupied half a thymic lobe. Such foci always involved the subcapsular region, and larger foci extended to the cortico-medullary junction (Fig. 2*a*); in such cases, donor type cells were always present in the underlying medulla. In 2 of 45 thymus lobes a striking repopulation was seen: a complete reconstitution of an entire thymic lobe by the minority repopulation of donor Thy-1 type cells (1:10 ratio, 2.5×10^6 total cells transferred), with only infrequent scattered individual cells of the host Thy-1 type. This type of reconstitution is identical to that seen in the allochimaeric thymus shown in Fig. 1. The data in Figs 1 and 2 demonstrate that one or a few cells can reconstitute an entire thymic lobe.

The most frequently observed pattern of donor type cells was that of focal accumulations of scattered cells present either individually (19 of 45 thymus lobes), or as a cluster of 2–5 cells

each (13 of 45 thymus lobes), generally present in both the outer and the deep cortex (Fig. 3); the medulla underlying areas of cortex containing these scattered cells also contained scattered donor type cells. We interpret these clusters and individual cells as representing the progeny of thymus homing cells of more limited proliferation potential, and perhaps of greater frequency than the fully reconstitutive thymic repopulating cells.

These various repopulation patterns could be seen as early as 13 days and as late as 10 weeks following irradiation and reconstitution. Thus we believe that we are seeing fundamental types of thymic reconstitution by incoming bone marrow cell types, and not a chance observation.

While it is possible that reconstitution occurred from multiple Thy-1 identical precursors by Thy-1 specific recruitment, preliminary experiments using bone marrow cells expressing Lyt-1 allotypic differentiation antigens also resulted in focal repopulations in three independent cases out of eight tested. We therefore conclude that these foci do not result from a Thy-1-allotype-specific congregation of cells but derive from single precursor cells.

A striking and entirely unexpected type of reconstitution occurred in 6 of the 45 thymus lobes examined: donor type thymocytes were identified only in the medulla (Fig. 2*a*, lobe on the right, and Fig. 2*b*). Multiple levels cut through these thymuses failed to demonstrate more than rare individual donor type thymocytes in the cortex. The donor type thymocytes comprised 40–60% of medullary thymocytes, and were scattered individually without clustering or localization to any specific region of the medulla. Host type thymus lymphocytes, present in abundant numbers in the cortex of these thymus lobes, made up the remainder of medullary thymic lymphocytes. While it is conceivable that medullary reconstitution could derive from recirculating mature T cells, the presence of medullary reconstitution in only one of two lobes (Fig. 2*a*) is not consistent with this pattern. All six lobes which exhibited this pattern of medullary reconstitution were from mice which had received relatively low absolute numbers of donor type bone marrow cells (1:50 ratio, 2.5×10^6 total cells transferred), and, like most thymuses, were examined 4 weeks after the transfer. Repopulation restricted to the medulla can be discerned only if there is no cortical repopulation in the same lobe by donor cells; its occurrence at higher doses of bone marrow cells may be obscured by concomitant cortical repopulations.

These findings support the proposition that the medulla may contain a separate lineage of cells which are capable of self-renewal over long intervals^{13,18,19}. However, it is not possible to rule out their derivation from cortical precursors although such cortical precursors would have to be very transient and/or very infrequent. Thus, in addition to the previously described cortex to medulla pathways of development of medullary thymocytes^{11,12}, there could be a separate lineage of medullary cells which does not involve substantial contribution from cortical precursors in mice. Such a pathway has been reported in birds¹⁸. It is not clear from these experiments if the cell which is capable of repopulating the medulla is different from the cell which is capable of repopulating the cortex to medulla pathway.

The peripheral T-cell population represents the full diversity of antigen-specific and MHC-restricted cells in each functional subclass, and it is important to define the stage of thymic maturation at which such diversity is generated. We¹ and others¹⁷ have postulated that this event could occur pre-thymically. However, experiments demonstrating oligoclonal repopulation of the thymus would seem to speak out against that possibility. However, most bone marrow chimaeras are created by the injection of 2×10^6 to 2×10^7 cells; by our calculations, these inocula would contain hundreds of clonogenic thymus-repopulation cells. Whether the full diversity of the T-cell repertoire, as well as its commitment to different functional lines, could be realized pre-thymically even with a few hundred clones is still in question.

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Accumulation of a heat shock-like protein during differentiation of human erythroid cell line K562

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The human erythroid cell line K562 provides a model system for studying erythroid differentiation and eukaryotic gene regulation. These cells express glycophorin A, spectrin and i antigen¹⁻⁴. They accumulate embryonic and fetal haemoglobins on induction of erythroid differentiation with haemin, sodium butyrate or hydroxyurea^{5,6}. In the present study, the protein composition of K562 cells during haemin-mediated induction of erythroid maturation was analysed by two-dimensional gel electrophoresis. Under conditions in which haemin did not affect cell viability and proliferation⁷, a protein of ~70,000 molecular weight (MW) accumulated in the differentiated K562 cells. The accumulation appears to be due to an increase in the rate of RNA synthesis for this protein. The protein is related in sequence to a 70,000-MW heat shock protein. An antigenically related protein was also demonstrated in human bone marrow and accumulates at particular stages of human erythroid maturation.

The total protein composition and rates of protein synthesis before and after haemin induction of K562 cells were examined (Fig. 1). After 40 h of incubation with 25 μ M haemin, approximately 95% of the K562 cells stained positively with benzidine. The two-dimensional electrophoretic analyses (Fig. 1) indicate that a 70,000-MW protein(s) (p70) accumulated in the haemin-induced sample (b), but not in the uninduced cells (a). Most other proteins were similar in both induced and uninduced cells. The similarity between isoelectric variants of p70 was demonstrated by two-dimensional peptide mapping analysis (data not shown). Figure 1d, e also shows that when cells were labelled with ³⁵S-methionine after induction with haemin, an enhanced labelling of the p70 occurred. We also used a cell-free system containing mRNA from haemin-treated K562 cells to show that the p70 protein represented a predominant peptide product which was not detectable among the translation products of control mRNA from untreated cells (Fig. 2). Therefore, the induction of this protein seems to be due to an accumulation of its mRNA over other cellular mRNAs.

As some heat shock stress proteins have been recently assigned a developmental role⁸⁻¹⁰, the response of K562 cells to heat shock was investigated. Figure 1c shows that after exposure to 43 °C, K562 cells accumulated a series of 70,000-MW heat shock protein(s). In addition to the similarity of molecular weight and

appearance in a two-dimensional gel, the heat shock p70 and the haemin-induced p70 show similarity in sequence as deduced from their similar peptide mappings described in Fig. 3 and from the digestion patterns of the two ³⁵S-labelled proteins by *Staphylococcus* protease¹¹.

An antigenic similarity between haemin-induced p70 and HeLa cell heat shock p70 was discovered by using antiserum to the HeLa cell proteins (given by J. Nevins). Cell extracts from control and haemin-induced K562 cells were separated by SDS-polyacrylamide gel electrophoresis (PAGE) and the proteins electro-blotted to nitrocellulose paper¹². Using these blots, antiserum against HeLa heat shock p70 reacted with 70,000-MW protein(s) from the K562 cells and these protein(s) were accumulated more than 10-fold in the induced cells compared with uninduced cells (Fig. 4c, d). The cross-reactive p70 in the haemin-induced K562 constituted approximately 6% of the total Coomassie-stained proteins (Fig. 4b). Given the immunological relatedness of haemin p70 to heat shock p70, we used a cDNA clone encoding the heat shock p70 in HeLa cells (a gift from J. Nevins) to assay the transcription rate of nascent RNA synthesis for its related protein. By using an *in vitro* nuclear transcription assay¹³, it was estimated that there was a ~5-fold increase in the rate of transcription for p70 protein(s) in haemin-induced cells (data not shown). Therefore, transcriptional stimulation seems to be responsible for the accumulation of mRNA for haemin p70 in the induced K562 cells.

An antigenically cross-reactive protein was detected in extracts of human bone marrow, but not in mature erythrocytes (see Fig. e, f). Specific immunoprecipitation of a 70,000-MW protein(s) was found (Fig. 4g, h) when extracts of ³⁵S-labelled bone marrow cells were precipitated with the same antiserum against heat shock p70, but not with control serum. Furthermore, when erythrocytes and late erythroblasts in bone marrow samples were lysed by hypotonic shock¹⁴, the 70,000-MW antigen reactive with anti-heat shock p70 was found only in the lysate and not in the pellet. This indicates that the antigen is present only in these relatively mature and lysable erythroid cells. In addition, when bone marrow cells were fractionated in a Percoll density gradient¹⁵, the ³⁵S-labelled p70 was present in the fraction, containing predominantly erythroblasts. Therefore, human bone marrow cells, probably the late erythroblasts, have protein(s) of molecular weight 70,000 that cross-react with anti-heat shock p70.

As haemin-induced p70 accumulated in the differentiated erythroblasts of both K562 and bone marrow, its induction in K562 cells may reflect a differentiation event, rather than being a general response to stress conditions. The inducer, haemin, is a normal cellular metabolite, important for normal regulation of protein synthesis and erythroid differentiation^{16,17}. The induction conditions applied did not affect cellular viability and proliferation⁷, and other stress conditions such as glucose starvation did not induce accumulation of the protein in K562 cells (unpublished data). Furthermore, haemin induction of the p70 exhibits tissue specificity, as incubation of HeLa cells with haemin did not result in production of a similar protein (unpublished observation). Recently, other heat shock proteins have been found to have a developmental role. Heat shock proteins 22 and 26 of *Drosophila* are differentially transcribed during normal development⁸. The expression of four small heat shock proteins in *Drosophila* marginal disk is stimulated by ecdysterone hormone⁹. Moreover, several genes in *Drosophila* and yeast, which are related to heat shock p70, but present at different chromosomal loci, are normally expressed during development but not induced by heat shock (the heat shock-cognate genes¹⁰). The haemin-induced p70 protein may represent a selective activation of gene(s) 'cognate' to the heat shock genes during erythroid maturation and independent of heat shock treatment.

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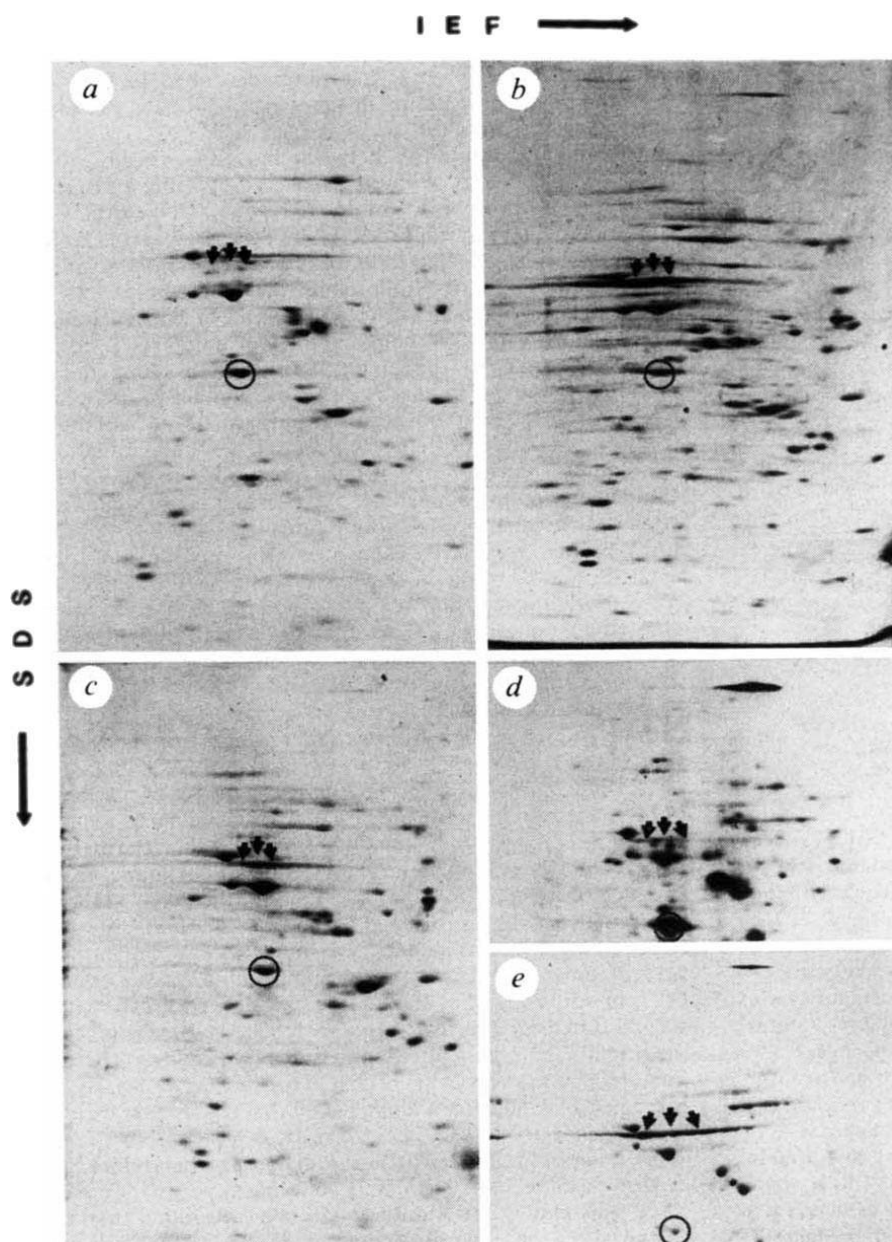


Fig. 1 Protein composition of: *a*, control; *b*, haemin- and *c*, heat shock-induced K562 cells analysed by two-dimensional gel electrophoresis. Arrows indicate the position of the 70,000-MW protein(s) and circles indicate actin. *d*, *e*, Fluorographs of the two-dimensional gels from samples of ^{35}S -labelled control and haemin-induced K562 cell, respectively. The cells were washed with Hank's balanced salt solution and labelled for 30 min in methionine-free RPMI (Gibco) medium containing 5% dialysed fetal calf serum and $50\ \mu\text{Ci ml}^{-1}$ ^{35}S -methionine. The fluorograph for haemin-induced cells was underexposed to show the enhanced synthesis of protein(s) indicated by arrows. Only those portions of the fluorographs with molecular weights larger than actin (indicated by circles) are shown.

Methods: K562 cells were grown in minimum essential medium containing 15% fetal calf serum and induced with $25\ \mu\text{M}$ haemin for 40 h. Heat shock treatment included incubation of K562 cells at 43°C for 1 h. The two-dimensional gel electrophoresis was done according to O'Farrell¹⁸. Briefly, 10^8 cells were washed with phosphate-buffered saline (PBS) and solubilized in $250\ \mu\text{l}$ of lysis buffer (9.5 M urea, 2% Nonidet P-40, 4% pH 5–7 ampholine, 1% pH 3–10 ampholine and 5% 2-mercaptoethanol) and $50\text{-}\mu\text{l}$ aliquots were applied to isoelectric focusing (IEF) gel containing the same ampholine concentrations. SDS-PAGE in the second dimension was carried out on a 7.5–15% acrylamide gradient gel¹⁹. The gels were stained with Coomassie blue as described elsewhere²⁰.

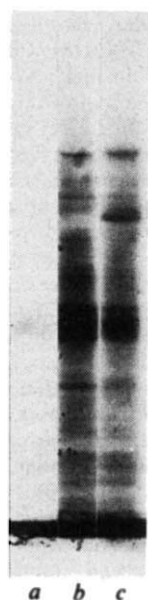


Fig. 2 *In vitro* protein synthesis directed by mRNA isolated from haemin-induced and uninduced K562 cells. RNA was isolated from uninduced K562 cells or cells induced in the presence of $25\ \mu\text{M}$ haemin for 48 h. After washing with PBS, the cells were solubilized in 4 M guanidine thiocyanate and RNA prepared by pelleting through a CsCl gradient as described²¹. The poly(A)-containing RNA was isolated by oligo(dT)-cellulose chromatography, translated in a rabbit reticulocyte lysate system and analysed by electrophoresis and fluorography. The sources of mRNA used in the *in vitro* translation are as follows: *a*, no mRNA; *b*, uninduced K562 cells; *c*, haemin-induced K562 cells.

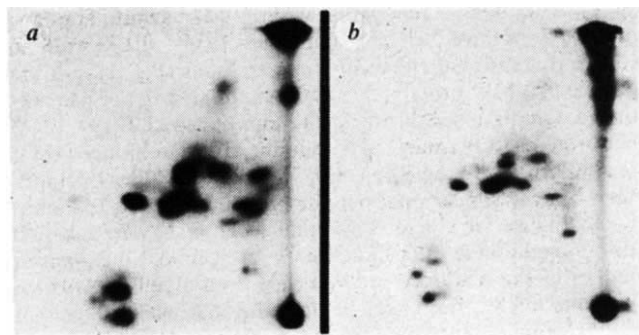


Fig. 3 Comparison of ^{125}I -labelled tryptic peptides of the haemin(*a*) and heat shock(*b*)-induced p70 of K562 cells. Gel slices were excised from the two-dimensional gel in Fig. 1 *b* and *c* containing the p70 proteins and radioiodinated as described²². After extensive washings, the proteins were cleaved with $5\ \mu\text{g}$ trypsin. Peptide analysis was performed by loading approximately 1×10^6 c.p.m. onto each cellulose-coated TLC plate. The samples were electrophoresed and chromatographed as described elsewhere²². Finally, the plates were dried and analysed by autoradiography.

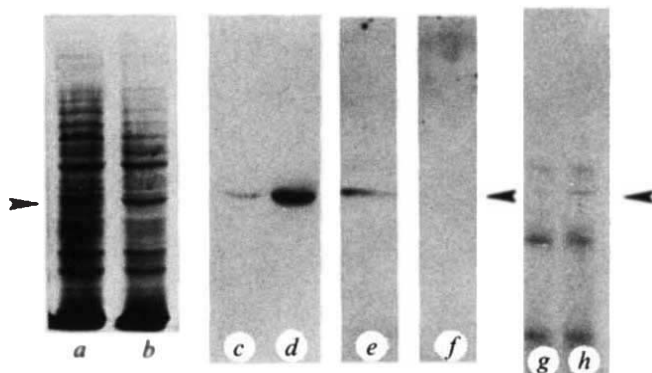


Fig. 4 Cross-reaction of a 70,000-MW protein in K562 cells and human bone marrow with antiserum against HeLa cell heat shock p70. K562 cells were induced with haemin as described in Fig. 1 legend. Equivalent aliquots of control (a, c) and induced (b, d) cells were washed extensively, solubilized by boiling in SDS-lysis buffer (62.5 mM Tris-HCl pH 6.8, 2% SDS, 10% glycerol, 5% β -mercaptoethanol and 0.001% pyronin Y), then analysed on a 7.5% acrylamide gel. Samples for e and f contained approximately 50 μ g of SDS-solubilized proteins from human bone marrow and adult erythrocytes, respectively. After electrophoresis, lanes a and b were stained with Coomassie blue and lanes c–f were electroblotted to nitrocellulose paper¹¹ and probed sequentially with antiserum against HeLa cell heat shock p70 and ¹²⁵I-labelled protein A. In other experiments, equal aliquots of the same cell extracts from the ³⁵S-labelled human bone marrow cells were immunoprecipitated (g, with control serum; h, with antiserum against heat shock p70). They were then electrophoresed in a 7.5–15% gradient gel containing 1% SDS. Arrows indicate the position corresponding to the 70,000-MW protein.

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Glycoprotein C of herpes simplex virus 1 acts as a receptor for the C3b complement component on infected cells

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Receptors for the Fc portion of immunoglobulins or for the third component of complement (C3) are present on a variety of circulating and fixed tissue cells including granulocytes, monocytes, lymphocytes and glomerular epithelial cells^{1–4}. Cells which lack Fc receptors may express them after infection by herpes simplex virus

(HSV)-1, HSV-2, cytomegalovirus or varicella zoster virus^{5–8}. We recently reported that infection by HSV-1 induces both Fc and C3 receptors on human endothelial cells⁹. Glycoprotein E of HSV-1 has been shown to function as an Fc receptor¹⁰. We now demonstrate that glycoprotein C (gC) of HSV-1 functions as a C3b receptor. This receptor appears following HSV-1, but not HSV-2, infection. Detection of the C3b receptor is blocked by monoclonal antibodies to glycoprotein C (gC) of HSV-1, but not by monoclonal antibodies to other HSV-1 glycoproteins. In addition, the MP mutant of HSV-1, which lacks gC, fails to express a C3b receptor. These results assign a new function of gC of HSV-1 and demonstrate potentially important differences between HSV-1 and HSV-2 glycoproteins.

Figure 1a shows the binding of C3b-coated erythrocytes to HSV-1 infected endothelial cells by light microscopy. Rosettes (>4 erythrocytes per endothelial cell) were found with 70% of infected endothelial cells: no rosettes formed around uninfected cells, nor around infected cells incubated with IgM-coated erythrocytes or with erythrocytes coated with complement components (C142) but not C3 (Fig. 1b). The specificity of the C3b-coated erythrocytes for C3b receptors was demonstrated by the ability of purified C3b fragments totally to inhibit rosetting. In contrast, purified C3 failed to block rosettes.

A second assay system was used to detect C3 receptors on infected endothelial cells (Fig. 1c). Complement-coated bacteria were prepared as indicator cells by incubating *Salmonella typhi* with antibody-negative fresh human serum¹¹. Extensive attachment of bacteria occurred to HSV-1 infected endothelial cells. No attachment occurred to uninfected cells. Similarly no attachment occurred to infected cells when bacteria were incubated with heat-inactivated serum rather than fresh serum as the source of complement.

These results suggested that the receptor might be a viral glycoprotein. To test this, endothelial cells were infected with HSV-1 in the presence of tunicamycin to inhibit glycoprotein expression^{12,13}. 3 μ g ml⁻¹ of tunicamycin added 1 h post-infection decreased from 70% to 5% the number of infected cells showing rosettes of C3b-coated erythrocytes at 24 h. Figure 2a shows the results expressed as the % binding of ⁵¹Cr-labelled, C3b-coated erythrocytes.

Experiments were performed with several different HSV-1 and HSV-2 strains. Four low passage clinical isolates of HSV-1 all induced C3b receptors (Fig. 2b). Light microscopy revealed rosettes of C3b coated erythrocytes around 70–75% of the infected endothelial cells following infection with each HSV-1 strain but no rosettes were found with clinical isolates of HSV-2 by either the ⁵¹Cr-erythrocyte binding assay (Fig. 2c) or microscopic examination. All HSV-1 and HSV-2 strains induced IgG Fc receptors as measured by binding and rosetting of IgG-coated, ⁵¹Cr-labelled erythrocytes (results not shown).

Glycoproteins A/B, D and E of HSV-1 have antigenic determinants in common with the equivalent glycoproteins of HSV-2 but gC of HSV-1 is predominantly type-specific^{14–16}. To examine whether gC acts as the C3b receptor, we looked for expression of the receptor in cells infected with the MP mutant of HSV-1, which fails to express gC¹⁷. No C3b receptor activity was detected in these cells, as measured by the ⁵¹Cr-erythrocyte binding assay (Fig. 2c) or by rosetting. In contrast, expression of Fc receptors by the MP mutant was normal, as detected both by the ⁵¹Cr-binding assay and by rosetting of IgG-coated erythrocytes (results not shown).

To define further the role of gC of HSV-1 as a C3b receptor, blocking assays were performed using a series of anti-herpes antibodies as described in the legend to Fig. 3. When HSV-1 infected endothelial cells were incubated with antiserum against HSV-1 envelope antigens¹⁵, binding of ⁵¹Cr-labelled, C3b-coated erythrocytes to infected cells was markedly reduced (Fig. 3). Monoclonal antibodies to gA/B, gD and gE had no effect on binding of these erythrocytes when antibodies were tested at a 1:10 dilution (Fig. 3) or undiluted (results not shown). In contrast, monoclonal antibody to gC reduced markedly the binding of ⁵¹Cr-labelled, C3b-coated erythrocytes. This reduction

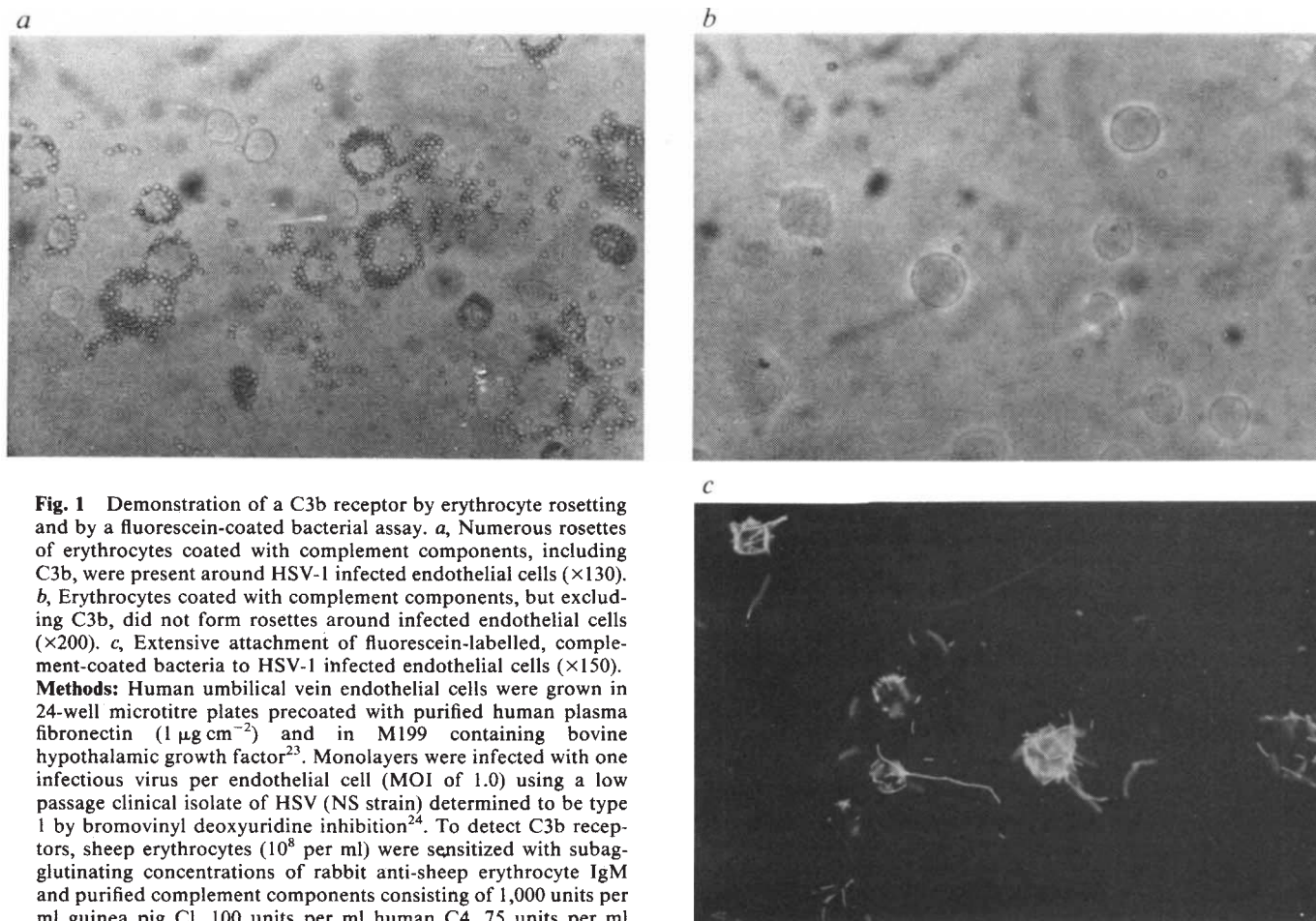


Fig. 1 Demonstration of a C3b receptor by erythrocyte rosetting and by a fluorescein-coated bacterial assay. *a*, Numerous rosettes of erythrocytes coated with complement components, including C3b, were present around HSV-1 infected endothelial cells ($\times 130$). *b*, Erythrocytes coated with complement components, but excluding C3b, did not form rosettes around infected endothelial cells ($\times 200$). *c*, Extensive attachment of fluorescein-labelled, complement-coated bacteria to HSV-1 infected endothelial cells ($\times 150$).

Methods: Human umbilical vein endothelial cells were grown in 24-well microtitre plates precoated with purified human plasma fibronectin ($1 \mu\text{g cm}^{-2}$) and in M199 containing bovine hypothalamic growth factor²³. Monolayers were infected with one infectious virus per endothelial cell (MOI of 1.0) using a low passage clinical isolate of HSV (NS strain) determined to be type 1 by bromovinyl deoxyuridine inhibition²⁴. To detect C3b receptors, sheep erythrocytes (10^8 per ml) were sensitized with subagglutinating concentrations of rabbit anti-sheep erythrocyte IgM and purified complement components consisting of 1,000 units per ml guinea pig C1, 100 units per ml human C4, 75 units per ml guinea pig C2 and 100 units per ml human C3 (Cordis Labs)^{9,25}.

Sheep erythrocytes were also prepared as above but C3 was omitted. Sheep erythrocytes without complement components were used in some experiments and prepared as described previously⁹. Erythrocyte rosetting assays⁹ were performed 24 h post-infection using a ratio of 100 erythrocytes per endothelial cell. Heat-killed *Salmonella typhi* were fluoresceinated and incubated for 15 min with human serum as a source of complement from a donor lacking antibodies to *Salmonella*.¹¹ 24 h after HSV-1 infection, endothelial cells were removed by scraping and were incubated for 30 min with complement-coated bacteria.

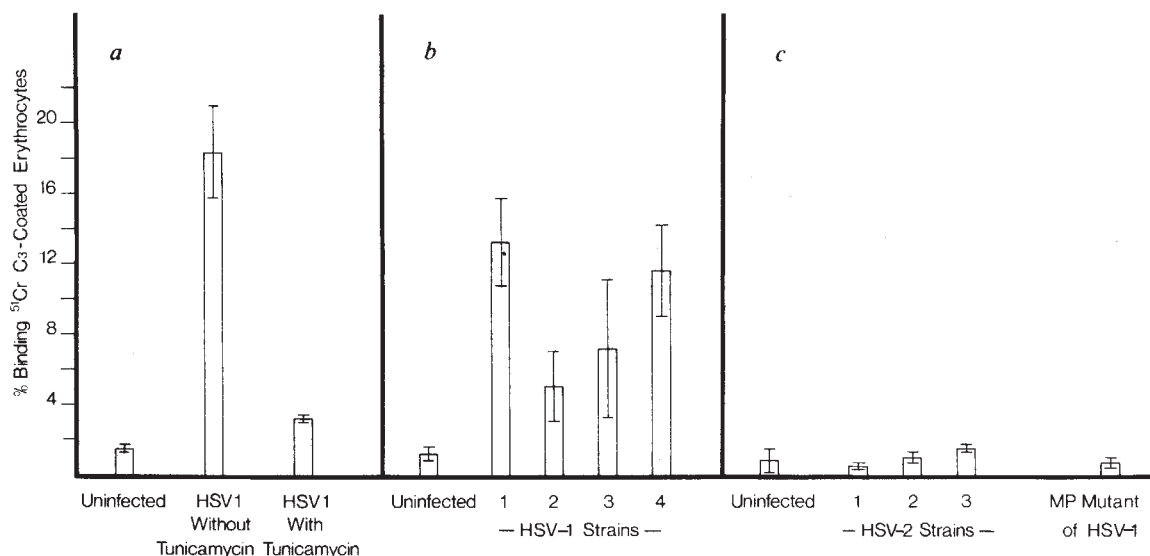
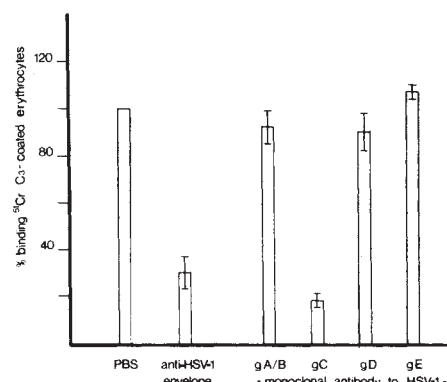


Fig. 2 Binding of coated erythrocytes to infected endothelial cells. *a*, Endothelial cells were infected with HSV 1 (NS strain) at an MOI of 1.0. One hour after infection, tunicamycin, $3 \mu\text{g ml}^{-1}$, was added to some cultures and 24 h after infection sheep erythrocytes coated with IgM and C1, 2, 3, 4 were added to detect C3b receptors. *b*, 24 h after infection of endothelial cells at an MOI of 1.0 with 4 HSV-1 low passage, clinical isolates, sheep erythrocytes coated as above were added to detect the C3b receptor. *c*, Three low passage HSV-2 strains and the MP mutant of HSV-1 were used at an MOI of 1.0 to infect endothelial cells. 24 h later, sheep erythrocytes coated as above were added to detect C3b receptors. At this time cytopathic changes were present in 100% of cells and 10^6 – 10^7 TCID₅₀ infectious viruses were being produced per culture well. Erythrocytes were radiolabelled with $100 \mu\text{Ci } ^{51}\text{Cr}$ ($\text{Na}_2^{51}\text{CrO}_4$) per 10^9 cells and prepared as described in the legend to Fig. 1. Binding assays were performed as described previously⁹ and expressed as the ratio of ⁵¹Cr-labelled erythrocytes bound to the monolayer to the total ⁵¹Cr-labelled erythrocytes added to the monolayer $\times 100\%$. Each bar is the mean of two experiments.

Fig. 3 Herpes antisera block the binding of C3b-coated erythrocytes to HSV-1 infected endothelial cells. Endothelial cells were infected with HSV-1 (NS strain) at an MOI of 1.0. 24 h later the monolayers were incubated for 30 min with 0.1 ml of either phosphate-buffered saline (PBS), antisera to HSV-1 envelope glycoproteins, or monoclonal antibodies to HSV-1 gA/B, gC, gD or gE. Unbound antiserum was removed by washing and ^{51}Cr -labelled erythrocytes, coated as described in the legend to Fig. 1, were added to detect HSV-1 induced C3b receptors. Binding of erythrocytes to infected cells in the presence of PBS was assigned a value of 100%. To calculate the effect of immune serum on blocking erythrocyte binding, the following formula was used:

$$\frac{{}^{51}\text{Cr-erythrocyte binding in presence of immune serum}}{{}^{51}\text{Cr-erythrocyte binding in presence of PBS}} \times 100\%$$

Monoclonal IgG antibodies to HSV-1, gA/B (24S), gC (5S, 17S, 19S, 27S) and gD (1S, HD1) were kindly provided by Drs B. Hamper, M. Zweig and L. Pereira. The specificity, neutralization titre, immunofluorescence activity and immunoglobulin subtypes of these monoclonal antibodies have been described elsewhere^{26,27}. Additional monoclonal antibodies were prepared by the method of Holland *et al.*¹⁴ against the low passage clinical isolate of HSV-1 (NS strain) used in these studies. Characterization of these antibodies as IgG2a anti-HSV-1 gC (1C8), gD (1D3) and gE (1E3) will be presented elsewhere (H.M.F. *et al.*, manuscript in preparation). Briefly, each of the monoclonal antibodies has neutralizing activity against HSV-1; the 1C8 antibody, however, requires complement for viral neutralization. The specificity of the monoclonal antibodies was demonstrated by radioimmunoprecipitation¹⁵ and SDS-polyacrylamide gel analysis of cytoplasmic extracts²⁸ prepared from HSV-1 infected Hep-2 cells labelled with ^{35}S -methionine 5–10 h post-infection. Each monoclonal antibody immunoprecipitated proteins corresponding in size to those published for precursor and processed forms of specific HSV glycoproteins^{10,28,29}. These were as follows: 1C8 anti-gC, 130,000 molecular weight (MW) and 105,000 MW; 1D3 anti-gD, 60,000 MW and 53,000 MW; 1E3 anti-gE, 80,000 MW and 68,000 MW. To block the binding of C3b-coated erythrocytes, a 1:10 dilution of ascites fluid was used. To demonstrate that binding to surface proteins occurs at this concentration, immunofluorescence studies were performed on uninfected HSV-1 infected and uninfected endothelial cells. The end-point titre was calculated as the highest dilution of antibody producing intense fluorescence on infected cells. The fluorescent antibody titres were as follows: anti-gB: 24S, 1:10; anti-gC: 17S, 1:1,000, 1C8, 1:1,000; anti-gD: HD1, 1:1,000, 1D3, 1:1,000; anti-gE: 1E3, 1:10. Each bar is the mean of two experiments except for the gC and gD monoclonal antibody experiments which are the mean of nine experiments.



occurred with each of the five anti-gC monoclonal antibodies examined at a 1:10 dilution (Fig. 3) and at a titre of 1:500 for the one anti-gC monoclonal antibody which was tested in serial dilutions (results not shown).

Anti-gC ascites (1C8) was purified by ammonium sulphate precipitation and DEAE-cellulose chromatography¹⁸. Purified IgG antibody (8.6 mg ml^{-1}) totally inhibited C3b-erythrocyte binding at a titre $\geq 1:1,000$. None of the anti-gC monoclonal antibodies, including purified 1C8 IgG, blocked binding of IgG-coated erythrocytes to the HSV-1 Fc receptor. The anti-gE monoclonal antibody blocked binding of IgC-coated erythrocytes to Fc receptors but had no effect on binding of C3b-coated erythrocytes to the C3 receptor. All these results were confirmed by rosettes, viewed under light microscopy.

These results indicate that gC of HSV-1, either alone or in combination with endothelial cell proteins, serves as a C3b receptor. Receptors for four different cleavage products of C3 have been described on haematopoietic cells, including receptors for C3b, C3b- $\beta 1\text{H}$, C3bi and C3d⁴. Whether any of these other C3 receptors are present on HSV-1 infected cells remains to be determined.

C3b receptors may serve as binding sites for complement-containing antigen-antibody complexes on certain fixed tissue cells and circulating blood cells^{19,20}. If the C3b receptors induced by HSV-1 function in a similar manner, they may bind these complement-containing complexes to infected endothelial cells leading to vessel wall injury. Furthermore, C3b receptors on erythrocytes have recently been shown to inhibit activation of both the classical and alternative complement pathways^{21,22}. If the viral induced C3b receptor also inhibits complement activation, the receptor may reduce the efficiency with which antibody and complement lyse infected cells but further studies are required to determine the importance of this glycoprotein in the pathogenesis of HSV-1 infection.

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Ultrabithorax mutations map to distant sites within the bithorax complex of *Drosophila*

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Chromosome breaks throughout a 70-kilobase (kb) region of the *Drosophila* bithorax complex (BX-C) result in the homeotic *Ultrabithorax* (*Ubx*) mutations¹. These cause the third thoracic and first abdominal segments of the *Drosophila* embryo to develop structures normally characteristic of the first and second thoracic segments^{2–6}. We show here by genetic mapping that apparent point mutations with *Ubx* phenotypes are located at both ends of this 70-kb region. Mutations located at either end of the *Ubx* unit have indistinguishable phenotypes, suggesting that both classes inactivate most or all wild-type, *Ubx*⁺, functions. This observation supports the suggestion¹ that *Ubx* mutations directly inactivate a product encoded by 5' and 3' exons of a long transcription unit.

Three *Ubx* mutations have previously been mapped by genetic recombination. All were found to lie at or close to the right-hand end of the *Ubx* unit defined by chromosome rearrangements^{1,7,8}. When the DNA of these and other *Ubx* 'point' alleles was examined, four apparently point *Ubx* mutations were found to be associated with small insertions or deletions¹. Three of these

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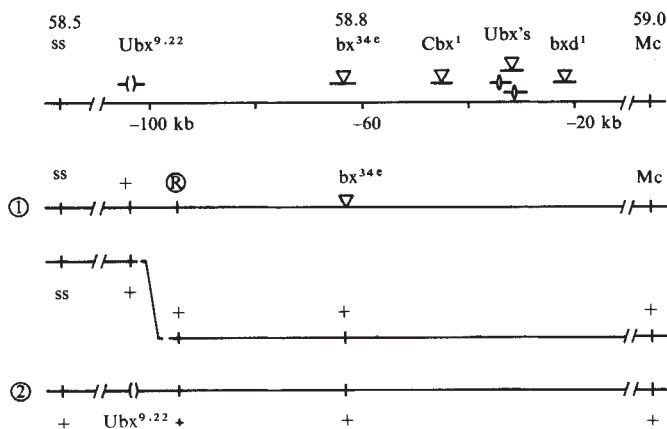


Fig. 1 Location of the *Ubx*^{9.22} mutation. Top: Map indicating the locations of relevant loci. Numbers at the top of the diagram give the standard genetic map positions of *ss* (*spineless*), *bx*^{34e} and *Mc* (*Microcephalus*) on the right arm of chromosome III. The DNA scale in kilobases shows the relative locations of insertions (∇) and deletions (—) in BX-C mutant chromosomes. The three *Ubx* lesions located close to -32 kb are *Ubx*¹ (insertion), *Ubx*^{6.28} and *Ubx*⁸⁴⁹ (deletions)¹. Bottom: Segregation of the *Ubx*^{9.22} mutant phenotype. The diagram shows the constitution of parentally derived chromosomes in non-recombinant progeny, and of one diagnostic recombinant. Plus signs indicate wild-type alleles at loci which differ between the parents. R is a restriction site polymorphism characteristic of the *bx*^{34e} chromosome. All wild-type recombinants lose both the *bx*^{34e} insertion and the *Ubx*^{9.22} deletion, and carry the *ss* flanking marker. In the diagnostic recombinant illustrated, recombination must have occurred to the left of -95 kb, as this recombinant chromosome carried restriction sites derived from the *Ubx*^{9.22} chromosome at the polymorphic site. From the phenotype of these recombinants in *trans* with *bx*^{34e} it remains possible that two separate mutations are responsible for the phenotype of the *Ubx*^{9.22} chromosome—a *bx* mutation located to the left of *bx*^{34e} and a *bxd* mutation located to the right. To eliminate this possibility, *bx*⁺ chromosomes from each recombinant were established in stock and examined as homozygotes, and in *trans* with a range of bithorax complex mutations, including the deficiencies *Df Ubx 109* or *Df Ubx P2* (ref. 4). In no case did these combinations reveal additional BX-C mutations remaining on the recombinant chromosomes. Thus, no DNA lesion to the right of -95 kb contributes to the phenotype of the *Ubx*^{9.22} chromosome.

lesions were located within a short (2 kb) target at the extreme right-hand end of the *Ubx* unit, within the region indicated by the genetic mapping. In the fourth case the only detectable lesion on the mutant chromosome was a 1.4-kb deletion located 70 kb from this site, at the extreme left end of the *Ubx* unit. The mapping crosses detailed below show that the *Ubx* mutation on this chromosome (*Ubx*^{9.22}) co-segregates with the observed deletion, and must be associated with DNA sequences to the left of coordinate -95 kb on the DNA map (Fig. 1), beyond the loci of *bx* (*bithorax*) and *Cbx* (*Contrabithorax*) mutations.

Table 1 gives the results of mapping crosses with *Ubx*^{9.22}, and with other previously unmapped *Ubx* alleles. Crosses with the *Ubx*^{6.28} mutation provide appropriate controls; *Ubx*^{9.22} and *Ubx*^{6.28} were X-ray-induced in the same experiment, on chromosomes of common origin⁹. In both cases, the mutant chromosome reveals a deletion when compared with the parental chromosome, but whereas the deletion in *Ubx*^{9.22} lies well to the left of the *bx* locus, that in *Ubx*^{6.28} lies in the expected target for *Ubx* 'point' mutations, to the right of *bx* and very close to the site of the *Ubx*¹ insertion¹ (Fig. 1).

Ubx^{9.22} and *Ubx*^{6.28} recombine with the *bx*^{34e} mutation at similar frequencies (Table 1). The flanking markers carried by wild-type recombinants indicate that the *Ubx*^{6.28} mutation maps, as expected, to the right of *bx*^{34e}. In contrast, wild-type recombinants with *Ubx*^{9.22} show the reverse segregation of flanking markers, confirming that the *Ubx* mutation maps to the left of *bx*^{34e}, and suggesting that it results from the observed deletion.

Table 1 Mapping *Ubx* alleles

<i>Ubx</i> allele	Cross type	Chromosomes tested	No. of BX-C ⁺ recombinants	Diagnostic flanking markers on recombinant chromosome	
				<i>ss</i> locus	<i>Mc</i> locus
9.22	1	10,500	7	<i>ss</i>	
9.22	2	10,400	4	<i>ss</i>	+
6.28	1	11,600	5	+	
98	2	7,600	4	+	<i>Mc</i>
154	2	13,500	4	+	<i>Mc</i>
159	2	28,000	4	+	<i>Mc</i>
195	2	22,000	0	—	—
847	2	17,000	3	+	<i>Mc</i>
849	2	13,600	3	+	<i>Mc</i>
859	2	13,000	3	+	<i>Mc</i>

Females heterozygous for each *Ubx* mutation, and for a chromosome carrying the *bx*^{34e} mutation in *cis* with flanking markers (see Fig. 1), were backcrossed to *bx*^{34e} homozygous males. Non-recombinant progeny are either *bx*^{34e}/*bx*^{34e} or *bx*^{34e}/*Ubx*. Both of these genotypes result in the partial transformation of the third thoracic segment into a second thoracic segment. Recombination between the *bx*^{34e} and *Ubx* mutations generates either wild-type (*bx*⁺ *Ubx*⁺) or double mutant (*bx*^{34e} *Ubx*) chromosomes. The *bx*⁺ *Ubx*⁺ haplotype in *trans* with *bx*^{34e} gives rise to phenotypically wild-type progeny, which we have identified in these crosses. Recombinants carrying the double mutant chromosome (*bx*^{34e} *Ubx*^{34e}) can be distinguished from the non-recombinant genotypes only with some difficulty; no attempt was made to identify such individuals in these crosses. The female genotype in crosses of type 1 was: *FMA3*, *XX/Y*, *SM1* *Cy/+*; *sbd*² *ss bx*^{34e}/*red sbd Ubx e*. The female genotype in type 2 crosses was *FMA3*, *XX/Y*; *+/+*; *In(3L)P*, *h sbd*² *ss bx*^{34e} *Mc/sbd Ubx*. The male genotype for all crosses was: *sbd ss bx*^{34e} (ref. 14). *Ubx*^{9.22} and *Ubx*^{6.28} have been described elsewhere⁹. The remaining *Ubx* alleles were recovered by Lewis after treatment of a *su(Hw)*² *sbd* chromosome with ethyl methane sulphonate; the allele numbers quoted are derived from the Lewis stock numbers. 98, 154, 159 and 195 are derived from Lewis cross 17756.98, 17756.154, 17756.159 and 17756.195; 847, 849 and 859 from cross 18136.147, 18136.149 and 18136.159 respectively. Heterozygous inversions in chromosomes I and II (series 1 crosses), or I and III left (series 2 crosses) were used to increase the frequency of recombination in the right arm of chromosome III. These enhanced recombination by a factor of ~3 (series 1 crosses, *ss-bx*^{34e} = 1.0% recombination) or 2 (series 2 crosses, *ss-Mc* = 1.0% recombination). Correcting for this, and for the recovery of only one recombinant class, the observed recombinants correspond to a map distance of ~0.04 from *bx*^{34e} to *Ubx*^{9.22}. The map distance between *bx*^{34e} and *Ubx* alleles located to the right (calculated on pooled data for *Ubx*s 6.28, 98, 154, 159, 849 and 859) is ~0.025 map units. The failure to observe recombinants with the *Ubx*¹⁹⁵ allele is significant when compared with these pooled data (expected = 5.5; observed = 0; *P* < 1%).

To define more precisely the limits within which the *Ubx*^{9.22} allele maps, DNA was prepared from strains homozygous for the recombinant chromosomes, and restriction fragments in the region of the bithorax complex were compared with those present in the *Ubx*^{9.22} and *bx*^{34e} stocks. In each of four tested cases, the recombinant chromosome lacked the restriction fragments characteristic of the gypsy insertion in *bx*^{34e} at -63.5 kb and the deleted fragments characteristic of the *Ubx*^{9.22} chromosome at -104 kb¹ (Fig. 1). In one particularly informative case (Fig. 1), the wild-type recombinant retained restriction fragments characteristic of the *Ubx*^{9.22} chromosome throughout the region from -30 to -95 kb, indicating that the recombination event occurred within 5 kb of the deletion present in the *Ubx*^{9.22} chromosome. We conclude that the *Ubx*^{9.22} chromosome carries no detectable BX-C mutation located to the right of coordinate -95 kb.

Although the *Ubx*^{9.22} mutation maps 70 kb from the sites of the *Ubx*¹ and *Ubx*^{6.28} mutations, the phenotypes resulting from all three of these mutations are virtually indistinguishable. Each shows the same range of phenotypes when in *trans* with *bx*, *bxd* and *pbx* (*postbithorax*) mutations of the BX-C^{9,10}. Homozygotes for each allele die as larvae, and those that survive to the third larval instar show partially developed anterior spiracles in the

third thoracic and first abdominal segments, which are characteristic of *Ubx* homozygotes⁴.

Similar mapping crosses were performed with a series of seven other *Ubx* mutations (Table 1). One of these, *Ubx*⁸⁴⁹, is associated with a ~110-base pair (bp) deletion in the *Ubx* 5' target, but the remainder are candidates for true point *Ubx* mutations¹.

*Ubx*⁸⁴⁹ and five of the six point alleles map clearly to the right of the *bx*^{34e} mutation. Our data are consistent with the model that all of these alleles map to the same site as *Ubx*^{6,28}, approximately 0.025 map units from *bx*^{34e}.

*Ubx*¹⁹⁵ does not fit this model. We observed no recombinants between this allele and *bx*^{34e} (see Table 1). Thus, *Ubx*¹⁹⁵ is most likely to map at a site closer to the *bx*^{34e} mutation than these other alleles.

We do not know the precise location of most of the *Ubx* mutations which map to the right of the *bx*^{34e} locus, but in those three cases where the mutation has been associated with an identified lesion in the DNA, this lesion is located between -31.5 and -32.5 kb¹, suggesting the existence of a localized target for such mutations.

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The *Ubx*^{9,22} mutation identifies a second target for *Ubx* alleles, which must be located to the left of -95 kb. We have not proved that this *Ubx* mutation results specifically from the observed deletion, but the circumstantial evidence strongly suggests that this is so.

DNA sequences located both in the rightward *Ubx* target and in the region removed by the *Ubx*^{9,22} deletion are present in processed transcripts of the *Ubx* unit (R. Saint and D. Hogness, unpublished results; see also refs 11-13). Taken together with these molecular data, our results support the suggestion¹ that *Ubx* mutations directly affect the expression of a product (or products) encoded by sequences which span the entire 70 kb of the *Ubx* unit.

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Xenopus oocytes can secrete bacterial β -lactamase

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Most secretory proteins are synthesized as precursor polypeptides carrying N-terminal, hydrophobic sequences which, by means of a signal recognition particle (SRP)^{1,3}, trigger the membrane transfer of the polypeptide and are subsequently cleaved off. The signal sequences appear to be interchangeable between prokaryotes and eukaryotes^{4,5}. In bacteria, secretion only involves the crossing of a membrane⁶, whereas in eukaryotes the secretory process can be separated into two distinct phases: translocation across the membrane of the rough endoplasmic reticulum and subsequent intraluminal transport by processes involving vesicle budding and fusion⁷. Since secretory proteins must be distinguished from other soluble proteins destined for various sites in the reticular system, it is conceivable that eukaryotic secretory proteins possess additional markers distinct from the signal peptide to guide the polypeptide after its transfer through the membrane. Proteins are secreted at different rates from a eukaryotic cell^{8,34}, suggesting a role in intracellular transport for receptors with differing affinities for some topogenic features in secretory proteins. We have tested this possibility by introducing into the lumen of eukaryotic rough endoplasmic reticulum a prokaryotic protein which, by virtue of its origin, had not been adapted to the eukaryotic secretory pathway. We reasoned that secretion of the bacterial protein would indicate that after membrane transfer no topogenic signal(s) and corresponding recognition system(s) are required. We report here that this is indeed the case.

We have chosen the β -lactamase encoded by the *Escherichia coli* plasmid pBR322 as a model for a prokaryotic secretory protein and *Xenopus* oocytes as eukaryotic test cells. β -Lactamase is the major protein secreted into the periplasm of *E. coli* cells carrying pBR322 and inactivates ampicillin, thus conferring on the bacteria resistance to the antibiotic. *Xenopus* oocytes have been shown to be a surrogate system for the study of the secretion of eukaryotic proteins (see ref. 9 for a review) or the incorporation and transport of plasma membrane pro-

teins^{10,11}. These cells, when microinjected with foreign mRNA, usually process the translation products in the same manner as the original cell and direct them to the same sites. They would therefore seem suitable for the present purpose.

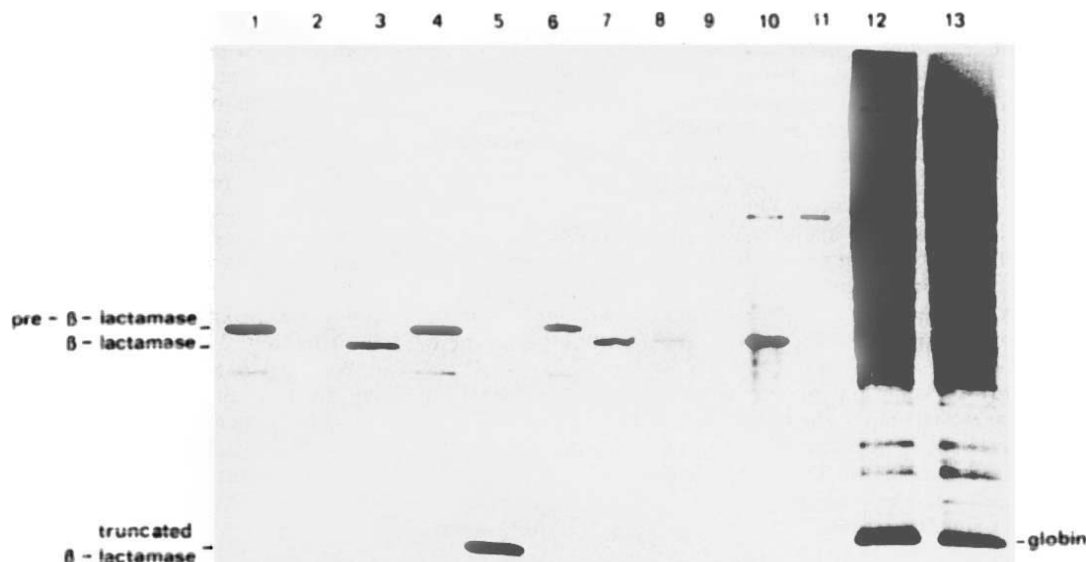
β -Lactamase-specific mRNA was synthesized *in vitro* using isolated RNA polymerase from *E. coli* and plasmid DNA as template¹². Capped mRNA was obtained by adding ⁷mGpppA to the transcription mixture¹³. Capped mRNA appears to be stabilized against the action of 5'-exonucleases (ref. 14, and data not shown) and is also more efficient than uncapped mRNA in eukaryotic translation systems¹⁵.

Translation of the *in vitro* synthesized RNA in the wheat germ cell-free system produced pre- β -lactamase (Fig. 1, lane 1). The *in vitro* transport of the prokaryotic polypeptide across the rough endoplasmic reticulum membrane was then tested. Addition of SRP, prepared from dog pancreas, to the translation assay abolished the synthesis of pre- β -lactamase (Fig. 1, lane 2). The translational arrest was prevented and signal peptide cleavage occurred if KCl-washed rough microsomes (K-RM) were also present during translation (Fig. 1, lane 3). These membranes are devoid of SRP¹⁶ and inactive by themselves (Fig. 1, lane 4). Identification of the β -lactamase was based on the observation that the product processed *in vitro* by the reconstituted translocation system co-migrated with the authentic enzyme in SDS gels (compare Fig. 1, lanes 3 and 7) and that a plasmid (pKT218) lacking the nucleotide sequence coding for amino acid residues 6-181 of pre- β -lactamase⁴ yielded a truncated translation product (Fig. 1, lane 5). Furthermore, cleavage with restriction enzymes within the β -lactamase gene prevented the synthesis of pre- β -lactamase after transcription-translation *in vitro*, whereas cleavage of the plasmid DNA outside the gene had no effect (data not shown).

These results are in complete agreement with those of Müller *et al.*⁵ and confirm that the prokaryotic signal peptide is recognized by mammalian SRP and is cleaved by the eukaryotic signal peptidase. Removal of the signal peptide of pre- β -lactamase has also been shown previously in yeast cells¹⁷.

As the wheat germ system possesses a capping system¹⁵, it was important to demonstrate the synthesis of pre- β -lactamase in a reticulocyte lysate system in which endogenous capping could not occur (Fig. 1, lane 6), as a precondition for the experiments with *Xenopus* oocytes in which only pre-capped mRNAs are efficiently translated^{14,18}. Synthesis was markedly

Fig. 1 Translation of *in vitro* synthesized β -lactamase mRNA in cell-free systems and in *Xenopus* oocytes. Lane 1, translation of pBR322-coded RNA in the wheat germ cell-free system. Lane 2, as lane 1 but in the presence of 5 units SRP. Lane 3, as lane 1 but in the presence of 5 units SRP and 0.5 equivalents of K-RM (for definition of units and equivalents see ref. 26). Lane 4, as lane 1 but in the presence of 0.5 equivalents of K-RM. Lane 5, translation of pKT218-coded RNA in the wheat germ system. Lane 6, translation of pBR322-coded RNA in the reticulocyte lysate system. Lane 7, ^3H -leucine-labelled periplasmic proteins of *E. coli* cells harbouring pBR322. Lane 8, products in the medium of four oocytes injected with pBR322-coded RNA and globin mRNA and labelled with ^{35}S -methionine (1 mCi ml^{-1} ; $1,100\text{ Ci mmol}^{-1}$) for 12 h (pulse). Lane 9, control to lane 8 with globin mRNA-injected oocytes. Lane 10, as lane 8 but after labelling with ^{35}S -methionine the incubation was continued for 22 h with new medium containing unlabelled methionine (10 mM) (chase). Lane 11, control to lane 10 with globin mRNA-injected oocytes. Lane 12, total products in pulse-chase labelled oocytes injected with pBR322-coded RNA and globin mRNA (the equivalent of 0.05 oocytes was applied). Lane 13, as lane 12 but oocytes only injected with globin mRNA.



Methods: β -Lactamase-specific mRNA was synthesized with isolated *E. coli* RNA polymerase and plasmid DNA as template. The assay contained in $10\text{ }\mu\text{l}$: 0.34 units *E. coli* RNA polymerase (PL Biochemicals), $3\text{ }\mu\text{g}$ of CsCl gradient-purified pBR322 DNA²⁷ or pKT218 DNA⁴, polyethyleneglycol 6000 (0.16 mg), RNase inhibitor from human placenta²⁸ (10^{-4} A_{280} units) and $^3\text{mGpppA}$ (5 nmol)¹³. Other conditions were as in ref. 5. After transcription the mixture was heated to 60°C for 5 min. Translation of $2.5\text{ }\mu\text{l}$ transcription mixture in a $12.5\text{-}\mu\text{l}$ assay of a micrococcal nuclease-treated wheat germ system²⁶ or of a nuclease-treated reticulocyte lysate system²⁹ was performed in the presence of ^{35}S -methionine (1 mCi ml^{-1}). SRP and K-RM were isolated from dog pancreas according to ref. 16. Proteins of *E. coli* cells carrying the plasmid pBR322 were labelled with ^3H -leucine for 60 min and the periplasm was prepared by lysozyme-EDTA treatment⁴. For the oocyte experiments, the *in vitro* synthesized RNA was extracted with phenol-chloroform (1:1), precipitated with ethanol and dissolved in water in $1/4$ of the original volume. $3\text{ }\mu\text{l}$ of this solution were mixed with $1\text{ }\mu\text{l}$ of rabbit globin mRNA (1 mg ml^{-1}) and 50 nl were injected into *Xenopus laevis* oocytes defolliculated with collagenase^{10,21}. Controls received 12.5 ng globin mRNA only. The oocytes were preincubated for 2 h before labelling. Incubations were performed in Barth medium³⁰ in the presence of penicillin ($10\text{ }\mu\text{g ml}^{-1}$) and protease inhibitors³¹. The translation products were separated on a 10–20% (w/v) linear acrylamide gradient SDS-gel³² and visualized by fluorography³³.

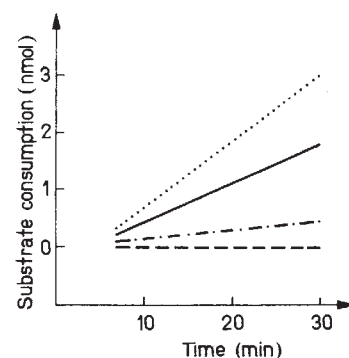
reduced in the reticulocyte lysate system with mRNA prepared in the absence of $^3\text{mGpppA}$ (data not shown).

β -Lactamase mRNA synthesized and capped *in vitro* was microinjected into oocytes and the translation products were labelled with ^{35}S -methionine. Globin mRNA was co-injected as an internal control. Globin was clearly seen among the products inside the oocyte, whereas the endogenous background did not allow a definite identification of a β -lactamase band (Fig. 1, lanes 12, 13). In the medium of oocytes incubated for 12 h with ^{35}S -methionine, a major band was seen at the position of β -lactamase (compare Fig. 1, lanes 7 and 8) which was absent from the medium of control oocytes (lane 9). The levels of β -lactamase in the medium increased after the chase period (lanes 10, 11), while globin was undetectable (lanes 8–11), excluding the possibility of nonspecific leakage from the oocytes.

Independent proof for the secretion of β -lactamase from *Xenopus* oocytes was obtained by measurement of the enzymatic activity in the medium. Nitrocefin was used as a sensitive chromogenic substrate of β -lactamase¹⁹ (Fig. 2). The medium of oocytes injected with plasmid-coded mRNA contained significant activity whereas globin mRNA-injected controls were negative. Again, in accordance with the labelling experiments, more β -lactamase was found after the chase period than before it.

Rough estimates of the number of enzyme molecules secreted by the oocytes could be obtained from both types of experiments. Using the radioactivity in the β -lactamase band and the methionine content of oocytes²⁰, it was calculated that 3×10^{10} molecules per oocyte were synthesized and secreted. Using enzymatic activity it was estimated that 10^{10} molecules of β -lactamase were secreted by one oocyte in 24 h (the specific activity of pure β -lactamase was assumed to be 200 units per mg^{17}). These values compare favourably with estimates of the amount of eukaryotic secretory proteins synthesized in and exported from oocytes^{10,21}. Also, the time periods between synthesis of the polypeptides and their appearance in the medium

Fig. 2 Detection of β -lactamase activity in the medium of oocytes injected with plasmid-coded mRNA. The β -lactamase activity was measured with nitrocefin as a chromogenic substrate (0.2 mg ml^{-1} in 50 mM phosphate buffer, $\text{pH } 7.0$) by the increase of the absorbance at 482 nm . The medium of two oocytes injected with pBR322-coded RNA and globin mRNA was tested after 12 h pulse-incubation (—) or after an additional 22 h of chase (—). A test with chase medium obtained from four oocytes (.....) showed nearly double the rate. Control oocytes received globin mRNA (---).



are similar (see ref. 22). Thus, the bacterial secretory protein may be handled in the eukaryotic cell with similar efficiency to a genuine eukaryotic protein.

Our results indicate that a signal peptide is sufficient to trigger secretion, even in a complex eukaryotic cell, and that no further topogenic information and corresponding recognition systems are needed after traversal of the membrane. Conversely, one may assume that non-secretory, soluble proteins, located in the lumen of the reticular system of a eukaryotic cell, need some device to prevent their secretion. For lysosomal enzymes of fibroblasts, for example, this mechanism appears to be the mannose 6-phosphate receptor pathway (for review see ref. 23).

Our results do not imply that every protein without a sorting signal will be secreted once it has passed the rough endoplasmic reticulum membrane. Poor solubility or special affinity to membranes can interfere with intraluminal transport (for examples see refs 24, 25).

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Sexual preference of apparent gene conversion events in MHC genes of mice

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Polymorphisms exist at many genetic loci. At some loci, however, polymorphism is so high that tens and even hundreds of different alleles coexist in the population. Two such highly polymorphic systems are the immunoglobulin genes and the vertebrate major histocompatibility loci. The origin and maintenance of highly polymorphic loci remain open to debate but it seems likely that special mechanisms contribute to their variability and that their polymorphism serves important biological roles. The high degree of polymorphism at the *H-2* class I major histocompatibility locus of the mouse has been documented by both tissue transplantation and serological methods¹. More recently, molecular cloning and DNA sequencing of some of the class I genes has shown that most of the sequence variability is concentrated in the first two domains and is often found in clustered regions within them^{2–4}. In addition, several groups have suggested that gene conversion events among the many class I genes may contribute to *H-2* polymorphism^{5,6}; such events would have to occur during meiosis to produce heritable alterations. The strongest evidence for gene conversion comes from sequence analysis of mutant class I *H-2* alleles where concerted changes at adjoining sites in DNA imply gene conversion by distant but closely related loci^{5,6}. We report here an analysis of these mutants indicating that the chromosomes containing loci that have experienced gene conversion originated from females. These data suggest a striking preference for mammalian meiotic gene conversion events during female rather than male gametogenesis.

The existing *H-2* mutants were originally identified by circular skin-grafting experiments among sibling mice^{7,8}. This system of

mutants is notable because: (1) the rate of observed mutation is very high, about 1 in 2,500; (2) individual mutational events result in multiple amino acid substitutions; (3) independent mutants have identical, multiple changes; and (4) in most cases, the newly introduced amino acid sequences are found in the same configuration in one of the other genes of the major histocompatibility complex (MHC). Two independent groups have recently shown unequivocally that in the case of the *bm1* mutant, a gene conversion-like event resulted in the substitution of a piece of DNA in the middle of the gene, producing the *K^{bm1}* gene^{5,6}.

While considering possible mechanisms for this high rate of gene conversion we were struck, as others had been previously⁹, by the apparent allele specificity of the conversion process. In the initial experiments, where skin grafts among (C57BL/6 × BALB/c)F₁ mice were performed, mutation was observed at the *K^b* locus to the almost total exclusion of alternative available loci (*K^d*, *D^b*, *D^d* and *L^d*). The results suggested that perhaps *K^b* is especially prone to alteration or possibly that changes in *K^b* were more easily recognized than others using a skin graft assay. The *K^b* gene, however, has no obvious uniqueness in its sequence and, since the initial experiments, mutations at other loci have been found. While puzzling over the existing data, a previously unrecognized feature became apparent. In the initial experiments, the F₁ mice all originated from mating C57BL/6 females with BALB/c males^{7,9–11}. Thus, the *K^b* allele was always contributed through the egg rather than the sperm. A review of all available data in which the origin of a mutated allele could be traced because the investigators used F₁ mice, showed that in every case, the mutation arose in a chromosome contributed by the egg (Table 1)^{7,8}. For this compilation, only mutants of the spontaneous gain/loss type were included because these are the ones most likely to involve a gene conversion-like event. (A gain/loss type mutant is one in which both the mutant and the parental type reciprocally reject skin grafts consistent with the simultaneous loss of one transplantation antigen and the acquisition of another.) There were 16 cases found, 9 of which involved *K^b*, 3 of which involved other *b* haplotype alleles and 4 of which were in maternal chromosomes from other haplotypes (Table 1). In one case, mutation in *K^d* was found in a strain where the *d* haplotype was maternal and the *b* haplotype paternal.

Gene conversion apparently occurred exclusively on maternal chromosomes in all cases examined. A maternal *K^b* allele was involved in many of these cases and if it had special properties then it would skew the data for reasons unrelated to the sex of the chromosome donor. Without the *K^b* events, however, all of seven cases were maternal, which would occur by chance with

Table 1 Gain/loss *H-2* mutants

Mutants	Affected locus	Maternal <i>H-2</i> haplotype	Paternal <i>H-2</i> haplotype	F ₁ cross in which mutation arose
<i>bm1</i>	<i>K^b</i>	<i>b</i>	<i>d</i>	C57BL/6By × BALB/cBy
<i>bm2</i>	<i>K^b</i>	<i>b</i>	<i>d</i>	C57BL/6By × BALB/cBy
<i>bm4</i>	<i>K^b</i>	<i>b</i>	<i>d</i>	C57BL/6By × BALB/cBy
<i>bm7</i>	<i>K^b</i>	<i>b</i>	<i>d</i>	C57BL/6By × BALB/cBy
<i>bm9</i>	<i>K^b</i>	<i>b</i>	<i>d</i>	C57BL/6By × BALB/cBy
<i>bm10</i>	<i>K^b</i>	<i>b</i>	<i>d</i>	C57BL/6By × BALB/cBy
<i>bm11</i>	<i>K^b</i>	<i>b</i>	<i>d</i>	C57BL/6By × BALB/cBy
<i>bm12</i>	<i>A^b</i>	<i>b</i>	<i>d</i>	C57BL/6By × BALB/cBy
<i>bm13</i>	<i>D^b</i>	<i>b</i>	<i>d</i>	C57BL/6By × BALB/cBy
<i>bm14</i>	<i>D^b</i>	<i>b</i>	<i>d</i>	C57BL/6By × BALB/cBy
<i>fm1</i>	<i>K^f</i>	<i>f</i>	<i>a</i>	A.CA/SnK1/Eg × A./Sn
<i>fm2</i>	<i>D^f</i>	<i>f</i>	<i>r</i>	B10.M × B10.RIII(71NS)
KH162A	<i>K^d-IA^d</i>	<i>d</i>	<i>b</i>	BALB/cKh × C57BL/6Kh
KH170	<i>K^b</i>	<i>b</i>	<i>d</i>	C57BL/6Kh × BALB/cKh
KH171	<i>K^b</i>	<i>b</i>	<i>d</i>	C57BL/6Kh × BALB/cKh
<i>dm5</i>	<i>K^d</i>	<i>d</i>	<i>b</i>	BALB/cKh × C57BL/6Kh

The origins of the mutants, together with the affected loci, are shown. For completeness, the table includes mutant *bm12*, which has a mutation in the *A^b* gene, and KH162 which has not been further characterized other than to be in the *K^d-IA^d* region. For details of the various origins of these mice see refs 7, 11.

a <1% probability. Although the rates of occurrence of gain/loss type mutants vary considerably, the events at K^b do not seem to be more frequent compared with other events in other haplotypes¹²; rather, K^b events are more frequent than D^b events. We conclude, therefore, that gene conversion events (if all MHC mutants have such an origin) are much more frequent in oogenesis than in spermatogenesis and possibly never occur during male gametogenesis. Although there are no available data for analysing any other gene system, it is reasonable to expect that in other multi-gene families, like immunoglobulin variable-region genes, the same specificity of gene conversion events might pertain.

Although gene conversion events in lower eukaryotic organisms are often associated with recombination of flanking

markers¹³, the localized gene conversion events at MHC and immunoglobulin genes are not known to have such an association. It may be relevant, however, that the intra- $H-2$ recombination rate is 0.32% in females and 0.19% in males¹². The excess rates in females are possibly associated with gene conversion events.

Although our analysis indicates preferential if not exclusive gene conversion in maternal gametogenesis, the preference of $H-2K$ conversion over $H-2D$ conversion still requires explanation⁷. Perhaps there is a polarization of events along the chromosome or some specificity associated with K^b structure.

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Nonspecific integration of the HTLV provirus genome into adult T-cell leukaemia cells

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Human T-cell leukaemia virus (HTLV)^{1–4}, previously also reported as ATL⁵, is a recently identified retrovirus which is closely associated with adult T-cell leukaemia (ATL) endemic in south-western Japan^{6,7} and the Caribbean⁸. Determination of the total nucleotide sequence of the HTLV genome has revealed no typical *onc* gene acquired from the cellular sequence^{9,10}. Screening of the HTLV provirus genome in tumour cells^{4,11,12} has shown that in all cases of ATL examined, the primary tumour cells contained the provirus genome and were monoclonal with respect to the integration site of the provirus¹³. These findings suggest that ATL leukaemogenesis may be due to insertional mutagenesis in which the provirus genome is integrated into a specific locus on the chromosomal DNA and then activates an adjacent cellular *onc* gene, a mechanism already demonstrated in avian lymphoma^{14,15} and erythroblastosis¹⁶ induced by avian leukosis viruses. A common site of HTLV provirus integration in leukaemic cells among some ATL patients was reported by Hahn *et al.*¹⁷ but subsequently retracted¹⁸. However, this retraction does not imply the random integration of the proviruses. Independently, we have been testing this insertional mutagenesis model in ATL and report here that the provirus did not have a common locus of integration in 35 ATL patients and did not integrate on the same chromosome in 2 ATL patients.

As previously reported, the leukaemic cells of most ATL patients contain one or two copies of HTLV provirus^{4,11}, but the size of the *Eco*RI-digested DNA fragment containing the provirus and cellular flanking sequences differs between individuals, suggesting that the integration sites of the HTLV provirus in these patients are not identical. However, even if the actual chromosomal integration sites are different, it is still possible that proviruses integrated into one or a few common regions could explain the activation of a unique cellular gene. The strategy used was to examine the rearrangements of cellular DNA sequences by provirus integration. By using specified

probes, we could detect the integration of provirus into any site within specified DNA fragments. We chose the DNA clone λ ATK-1 (ref. 10), which had been isolated from fresh leukaemic cells of an ATL patient (KKT). As these cells had only one copy of the provirus genome¹⁰, the provirus must be involved in initiating or promoting leukaemic cell growth. The cellular flanking sequence of this provirus could therefore be used as a chromosomal site-specific probe to test the significance of provirus integration into the tumour cell DNA.

The unique cellular sequence flanking the 3' long terminal repeat (LTR) was subcloned into pBR322 and used as probe *a* (Fig. 1a) for screening a human gene library to isolate clones covering wider regions around the provirus integration site. Isolation of three independent clones (GL1, GL2 and GL3) and construction of their restriction maps resulted in three *Sst*I fragments (A, B and C, Fig. 1A) covering a region of about 28 kilobases (kb) around the provirus integration site in leukaemic cell DNA of patient KKT. We subcloned the unique sequences *a*, *b* and *c* from fragments A, B and C, respectively, and used them as probes to detect DNA rearrangements in *Sst*I digests of leukaemic cell DNA from 35 other ATL patients. As *Sst*I does not cut the viral sequences, the integrated fragments with the HTLV provirus sequence should have higher molecular weights than the original fragments A, B and C. Figure 1B shows some results of screening using probe *a*. In addition to the normal cellular fragments, a rearranged larger fragment was detected in the control DNA of patient KKT from which the original clone was isolated, but no such rearranged fragments were detected in the DNA of the other ATL patients. These results were confirmed by rehybridization of the same filters using viral probes that detected co-migration of the only provirus sequence with the rearranged fragment of patient KKT (Fig. 1c). No DNA rearrangements were detected with probes *b* and *c* (data not shown). This absence of a common region for provirus integration in leukaemic cells suggests that it is unlikely that ATL is caused by simple insertional mutagenesis.

This conclusion is based on analysis of a single locus of a provirus integration in only one case of ATL, which could be an exceptional case. To exclude this possibility, we isolated another clone, λ ATY, from fresh leukaemic cells of another ATL patient (MYK). Blotting analysis showed that these leukaemic cells also contained only one copy of the provirus genome. The unique cellular flanking sequence in patient MYK was subcloned and used as a probe in the same way as described above. This probe (probe *d*) detected an 8-kb fragment in the *Hind*III digest of normal cellular DNA and a 10.5-kb rearranged fragment in the digest of DNA from patient MYK (data not shown). Again, probe *d* did not detect any rearranged fragment

Table 1 Distribution of probes *a* and *d* in somatic cell hybrids

	Probes		Chromosomes determined by enzyme markers																							
	<i>a</i> (KKT)	<i>d</i> (MYK)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X*	
Cell hybrids	—	+	—	—	—	—	—	—	—	+	—	—	—	+	—	—	—	—	+	—	—	+	+	—	—	
ICL-15	+	+	—	—	+	—	—	—	+	+	—	+	—	—	—	+	+	—	+	—	—	+	—	—	+	
WIL-14	+	+	—	+	—	+	+	+	+	+	—	+	+	—	—	+	—	—	+	—	+	+	+	—	+	
WIL-6	—	+	—	—	—	+	—	—	—	+	—	+	—	—	—	—	—	—	+	+	—	—	+	—	+	
WIL-5	—	+	—	—	—	—	—	—	—	+	+	—	—	—	—	—	—	—	+	+	—	—	+	—	+	
WIL-2	—	+	—	—	—	—	—	—	—	+	+	—	—	+	+	—	+	—	+	—	+	—	+	—	+	
WIL-1	—	+	+	+	—	—	—	—	—	+	+	+	—	+	+	+	—	—	+	+	—	—	+	—	+	
ATR-15	+ ^w	—	+	+	+	+	+	—	+	+	—	+	—	+	+	+	+	—	+	+	+	+	+	+	+	
ATR-13	+	+ ^w	+	+	+	+	+	+	+	+	—	+	—	+	—	+	+	+	+	+	+	—	+	—	+	
ATR-11	+	—	+	+	+	+	+	—	+	+	—	+	+	+	+	+	+	+	—	+	+	—	+	+	+	
NSL-9	—	+	—	—	—	—	+	—	—	+	—	—	—	+	+	+	+	+	+	+	—	+	+	—	—	
TSL-2	—	+	—	+	+	—	+	+	—	—	—	+	—	+	—	—	—	—	+	+	—	+	+	—	—	
XER-7	+	—	+	+	+	+	+	+	+	+	+	+	—	+	+	+	+	—	+	+	—	—	—	—	—	
EXR-5 CSAz	+ ^w	—	+	+	+	—	+	+	+	+	+	—	—	+	—	+	—	—	—	+	+	—	+	+	—	
% Discordancy for <i>a</i>			23	23	15	23	23	31	0	38	54	38	38	54	54	15	31	46	77	46	15	62	62	38	31	
% Discordancy for <i>d</i>			85	69	77	69	69	62	77	38	69	38	69	54	69	62	62	62	0	62	77	46	31	85	46	

Probe *a* (KKT), A in Fig. 3; probe *d* (MYK), B in Fig. 3. % Discordancy indicates the frequency that probes do not co-segregate with a specific human chromosome. Human chromosomes were determined by analysing each cell hybrid for enzyme markers encoded on each human chromosome as described previously²¹. +^w, weak band.

* Chromosome Y is not involved as human female parental cells were used.

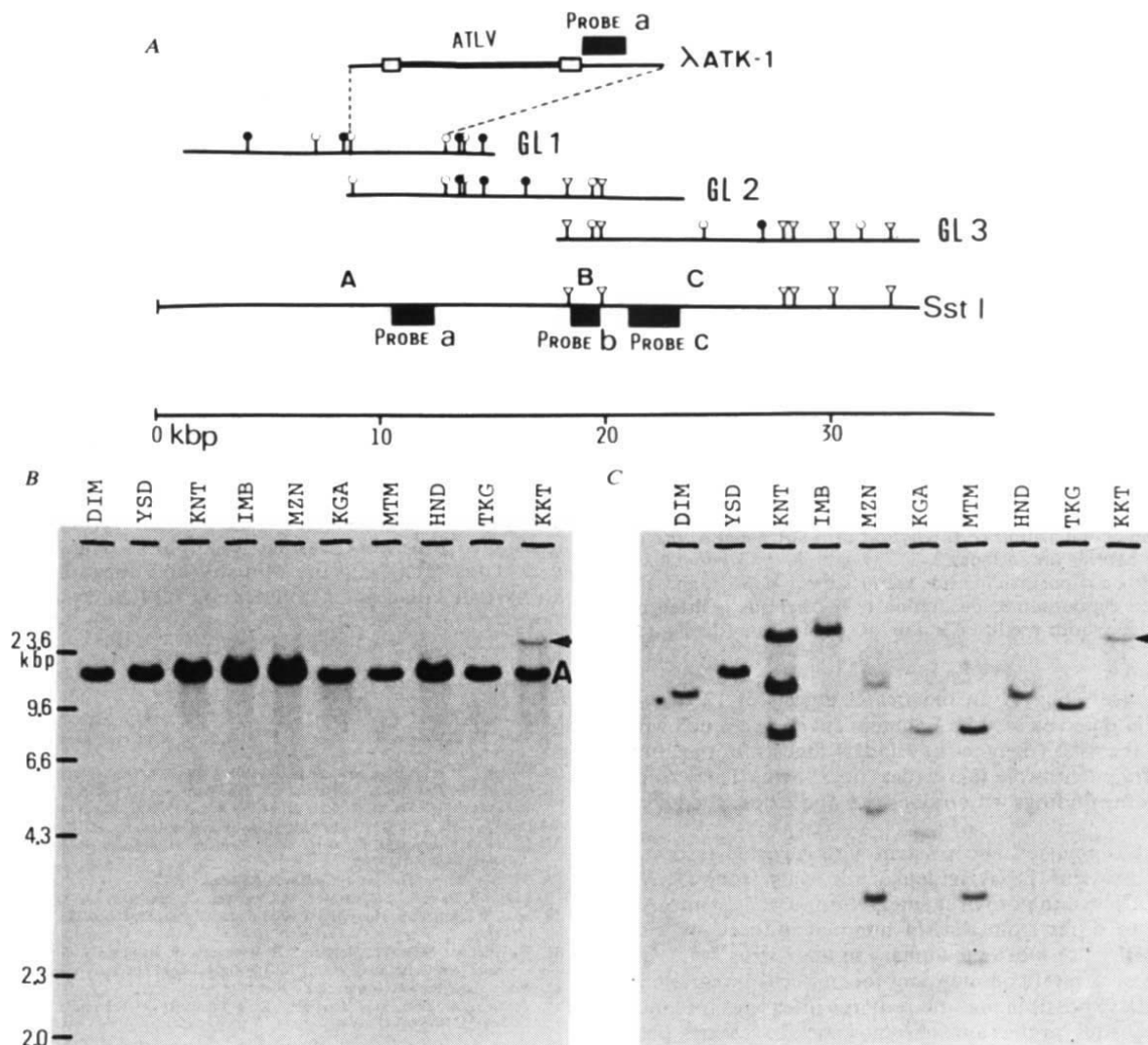


Fig. 1 Analysis of provirus integration sites in leukaemic cells from ATL patients using probes of cellular flanking sequences. **A**, Clones GL1, GL2 and GL3 of cellular DNA adjacent to the provirus genome integrated into leukaemia cells of a patient (KKT) were isolated from a human gene library using probe *a* and then probe *c*, and the restriction map was constructed. Probes *a*, *b* and *c* were separately subcloned into pBR322. $\square$, *EcoRI*; $\circ$, *HindIII*; γ , *SstI*. **B**, DNA samples from fresh leukaemic cells of independent ATL patients were digested with *SstI*, fractionated in 0.8% agarose gel and transferred to a nitrocellulose filter. The filter was hybridized with ³²P-labelled probe *a*. Lane KKT is DNA from which the original provirus clone λ ATK-1 was isolated, and other lanes are DNAs from other ATL patients. A indicates normal fragments detected by probe *a*. The fragment rearranged by provirus integration is indicated by an arrow. **C**, The filter used in **B** was washed in boiling water for 10 min and used for a second hybridization with ³²P-labelled HTLV representative probe.

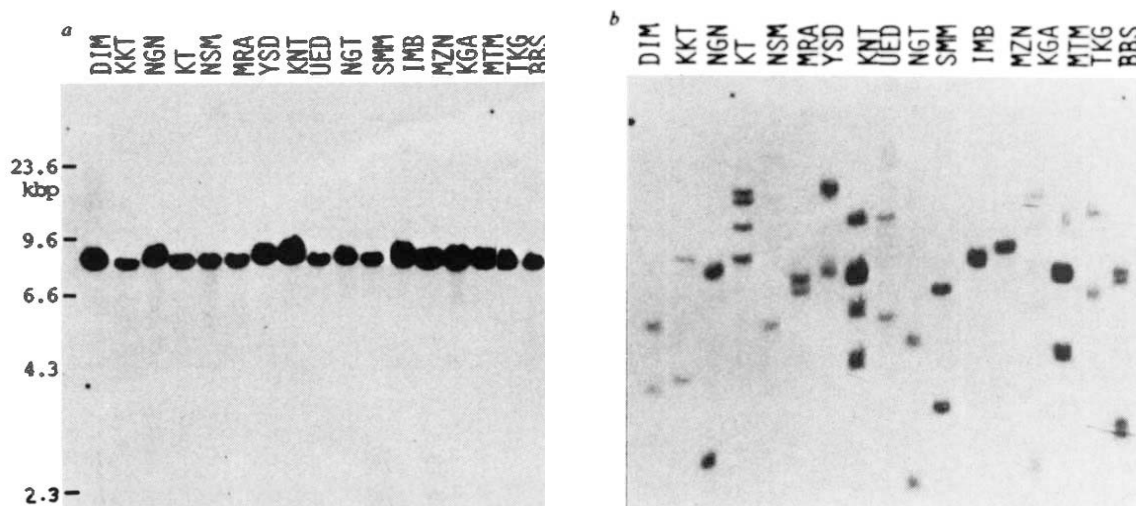


Fig. 2 Analysis of provirus integration sites in DNA of leukaemic cells from ATL patients with a second independent cellular flanking sequence. *a*, The filter was hybridized with ^{32}P -labelled probe *d*. *b*, The same filter was hybridized with ^{32}P -labelled HTLV as representative probe. Molecular clone λ ATY was obtained from an *Sst*I digest of DNA from another ATL patient (MYK) according to the methods reported previously⁹. The unique cellular sequence adjacent to 3' of the provirus was subcloned from the *Hpa*II digest of λ ATY into pBR322 and used as probe *d*. DNA samples from ATL patients were digested with *Hind*III and analysed as in Fig. 1.

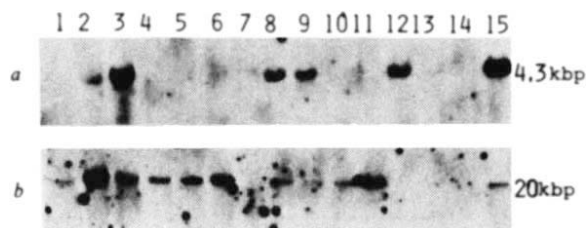


Fig. 3 Distribution of cellular sequences flanking adjacent integrated proviruses among human-mouse somatic hybrid cells containing different sets of human chromosomes (see Table 1). Hybridization of ^{32}P -labelled probes *a* (panel *a*) and *d* (panel *b*) to *Eco*RI-digested DNA from somatic cell hybrids (lanes 1–13), mouse (lane 14) and human (lane 15) cultured cells^{22–24} after DNA fragments had been separated by electrophoresis and transferred to nitrocellulose filter. Probe *a* hybridizes to a 4.3-kb fragment and probe *d* hybridizes to a 20-kb fragment but neither hybridizes to mouse in these conditions. Cell hybrid DNAs either contain the human sequence (+) or do not (–), depending on the human chromosome composition of the hybrid cells. In panel *a*, probe *a*-positive hybrids are in lanes 2, 3, 7 (weak), 8, 9, 12 and 13. In panel *b*, probe *d*-positive hybrids are in lanes 1–6, 8, 10 and 11. The human chromosome constitution of the hybrids is listed in Table 1; the hybrids are listed in the same order as in the figure.

in 17 ATL cases (Fig. 2). In the *Eco*RI digests of 12 cases of ATL, probe *d* detected a 20-kb fragment covering a much wider region than the 8-kb fragment in *Hind*III digests, but again no rearranged fragments were found (data not shown). These results also support the findings with probes *a*, *b* and *c* described above (Fig. 1).

The above conclusion is consistent with reported restriction patterns of provirus DNA in leukaemic cells from 78 ATL patients^{11,13}. Thus, the provirus integration of HTLV into ATL cells seems to differ from that in tumours induced by avian leukosis virus^{14–16} or mouse mammary tumour virus^{19,20}, which possess one or several common loci for provirus integration.

However, it is possible that the provirus integrates into many chromosomal sites on the same chromosome. To test this possibility, we analysed the specificity of provirus integration at the chromosomal level by using the somatic cell hybrid strategy for mapping human genes on specific chromosomes²¹. Human $\times$ mouse somatic cell hybrids characterized for their human chromosomal content were analysed for probes *a* and *d*. DNA was extracted from these hybrid cells and separately analysed by the two probes on the same filter (Fig. 3). The chromosomes in which the proviruses are integrated segregated independently

in cell hybrids (Table 1), demonstrating that integration occurs on different chromosomes. Analysis of the distribution of the probes in the cell hybrids tested in Fig. 3 demonstrates that probe *a* co-segregated with chromosome 7 and probe *d* co-segregated with chromosome 17 (Table 1). With the small number of cell hybrids tested, the evidence strongly suggests that the sequences recognized by probes *a* and *d* are located on chromosomes 7 and 17, respectively. Results from 15 additional hybrids tested similarly, support the chromosome mapping evidence in Table 1 (data not shown).

The absence of chromosomal specificity of the provirus integration between two ATL patients is consistent with the absence of a common integration site shown by blotting analysis of the provirus sequence in leukaemic cells. These data strongly suggest that provirus integration in tumour cells occurs at random sites.

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Father, mother and son

Peter Kemp

H.G. Wells: Aspects of a Life. By Anthony West.
Random House/Hutchinson: 1984. Pp.405. \$22.95, £12.95.

H.G. Wells: Aspects of a Life opens bizarrely not with the birth of its subject but the birth of the biographer — Anthony West, Wells's illegitimate son. At first startling, this emerges as rather appropriate, since, for most of the book, West's technique is to superimpose his own situation upon his subject-matter. Ultimately, it comes to seem, the aspects of a life being illuminated here are not so much Wells's but West's.

Even structurally, the book throws West into unexpected prominence — not only starting with his birth but ending at the moment when he resolved to write his father's life. The way he has gone about this is idiosyncratic, too. Most attention is devoted to the numerous women with whom Wells was involved, West's mother — the writer Rebecca West — attracting a particularly long and loathing commentary. Savage disparagement is further lavished on literary figures whose paths crossed Wells's, especially Henry James, George Bernard Shaw and George Gissing.

West's unusual handling of his material means that the real shape and substance of Wells's life get obscured — especially the trajectory he followed from a dismally disadvantaged background to fame as a novelist then descent into the role of wordy opiner on world affairs. Fuelling that progress was fierce energy. Born to parents just subsisting in a floundering little shop, Wells seemed destined — like his brothers — to life as a draper's assistant. What saved him was science. Angrily and admirably elbowing his way to an education, he eventually won a scholarship to the Normal School of Science. Here, he was taught by T.H. Huxley — who influenced him in two crucial ways. He instilled into him the importance of taking a biological view of life: something that gives Wells's writings — despite their surface diversity: fiction, Utopias, encyclopaedias, biography, autobiography, polemic and propaganda — an essential unity. And his career showed Wells what might be achieved by pugnacious advocacy of scientific training.

Initially, Wells's educational impulse took him into school-teaching. Ill health ended this. But, when he began writing, the

impulse stayed with him: didactic hypotheses are fruitfully yoked to imaginative excitements in the early scientific romances — *The Time Machine*, *The Island of Doctor Moreau*, *The Invisible Man*, *The War of the Worlds*, *The First Men in the Moon* and so on — that established him as an intriguingly new kind of creative figure.



Huxley's student — H.G. Wells at the Normal School in South Kensington.

Strangely, when it comes to conveying the nature of Wells's achievement, West concentrates on his inferior, post-1914 writing. Preferring the journalist to the novelist, he lauds the bombastic and ephemeral newspaper work turned out — indeed, churned out — when Wells was straining to establish himself as a kind of global guru. Given more perfunctory treatment is the period of real brilliance in Wells's career — his first two decades as an author: when he turned the dreary and debilitating events of his early life into bracingly resilient comic fiction, and channelled the intellectual energies generated by his studies with T.H. Huxley into powerful, pulsing science fiction. Confusing notoriety with notability, West appears

most impressed by the later Wells — “a world figure whose name was known wherever books were printed and newspapers were sold”, “a very big man indeed”.

Besides beating the drum for this strutting public figure, West is concerned with putting an attractive gloss on Wells's private affairs. It is here that his book becomes most extraordinarily personal. The idea of writing his father's life came to him, West reveals, when he was brooding over the fact that someone seemed to have lied to Wells about him, alleging that he belonged to a pro-Nazi conspiracy. Appalled at “The wilful interpolation of a piece of fiction into the facts of my life”, West was

struck with horror at the thought that just such another “nightmare cobweb of lies” might be draped across Wells's biography. Accordingly, he resolved “to devote the necessary time and effort required to keeping the record of what my father had been, and of what he stood for, more or less straight”.

Crooked records are an *idée fixe* in this book. West finds doctored documents everywhere. His grandmother apparently concocted an “appalling fabricated diary”. His mother, he claims, bequeathed to posterity a trumped-up tissue of falsifications about her relationship with Wells — drafts or summaries of letters that never existed, invented incidents, fake quotations. Another woman novelist who became Wells's mistress, Dorothy Richardson, is likewise sourly indicted of compulsive mendacity about their affair. Even a male friend of Wells's, the novelist George Gissing, gets denounced as a “veiled and secretive fraud”, an obsessive “fabulist” for whom “the transformation of the truth was the name of the game”.

All of this needs viewing sceptically. It is, it transpires, a habit of West's to assail people for doing what he does himself: at one point, with the shattering imprudence of the stone-thrower in the glass-house, he even lobs abuse at someone for “producing studies of the lives of dead writers”. Regularly, he protests about the snobbery often shown towards Wells — while at the same time indulging himself in a very socially snuffy tone, turning up his nose at “provincials who lived in a provincial backwater”, “second-raters” in “the obscurer colleges of the older universities”, and Dorothy Richardson's “not very dazzling receptionist's job”. Constantly, people are rapped over the knuckles for taking hearsay on trust. Yet this is West's habitual procedure: most of his story of Gissing's relations with his se-

From H.G. Wells: *Aspects of a Life*

cond wife, Edith, for instance, consists of West's account of Wells's account of a doctor's account of Gissing's account of his dealings with Edith — the resulting hodge-podge being, unsurprisingly, grotesquely at variance with Gissing's direct record of events in his diary. Quotations from Gissing's letters are scrambled too — up to five distortions in six lines — giving a misleadingly unpleasant slant to things: he's made to refer to his son as "it" not "he", his wife as "the woman" not "the poor woman".

What seems to be happening here is that facts about Gissing's life are being slid around until they fall into a pattern fitting West's preoccupations. Ferociously, West clamps the complex of feelings stemming from his lonely and divided childhood around other people. In his *Experiment in Autobiography*, for instance, Wells recounts how as a boy, when tossed playfully into the air by a youth teasing him with "Whose little kid are you?", he fell and broke his leg. This was, he observes, actually a lucky break: confined to bed and plied with delicacies and books by the youth's mother, he acquired that appetite for reading and knowledge that, via his exhilarating educational experiences studying biology with Huxley, eventually launched his career: "Probably I am alive to-day and writing this autobiography instead of being a worn-out, dismissed and already dead shop-assistant, because my leg was broken". West's interpretation of events is strikingly different: the youth, he believes, "had really put him on the spot with his question: Whose little kid was he, his father's or his mother's?"; he "had been asking him to take sides" and "The probability is that he had been trying to blurt out his father's name when he was dropped", so "It must have seemed as if he had been punished for wanting to be his father's boy".

There is, in fact, nothing in Wells's account to warrant the assertion that he was being asked which parent he preferred, or that he "had been terribly torn between being his mother's boy or his father's". West, however, has spent his life caught in the tensions of such a pull — as his novel *Heritage* about his filial fight against Rebecca West makes only too clear. His new book seems one more round in the psychic tug-of-war between what he calls in a sadly revealing phrase "the father of my dreams", and the mother whose accounts of other people "however sharply detailed and however plausible in their *trompe l'oeil* literalness, are expressive of her states of feeling rather than descriptions of actual . . . occurrences". Ironically, on the evidence presented here, West establishes himself not so much as his father's champion as his mother's son. □

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Processes of natural waters

J.D. Burton

Principles of Aquatic Chemistry.

By François M.M. Morel.

Wiley: 1983. Pp.446.

£42.55, \$49.95.

THE study of chemical processes in natural waters tends to be pursued in compartments — oceans, lakes, rivers, groundwaters and so on. One reason for this lies in the large differences between such environments regarding the spatial and temporal frameworks within which geochemical processes can operate. Another is the contrast in ionic strength between saline and fresh waters, which to some extent imposes different strategies upon the application of both analytical techniques and physicochemical concepts.

Nevertheless, underlying the diversity of geochemical phenomena in natural aquatic systems is a limited number of physicochemical processes which also underlie the chemical behaviour of constituents in treated waters and aqueous wastes.

Professor Morel's textbook is the latest of several which have presented the chemistry of natural aquatic environments in the context of basic physicochemical principles. The first three chapters give a concise and clearly developed introduction to solution chemistry. The subsequent treatment of natural water chemistry is organized along lines dictated by the main

types of reactions which have to be considered: acid-base chemistry, solubility equilibria, complexation, redox chemistry and surface chemistry. The tone is firmly didactic and numerical examples are widely used. Any such approach must be rooted in equilibrium systems but the importance of kinetic factors in determining what happens in the environment receives appropriate emphasis. The dominance of inorganic topics largely reflects the relatively backward status of attempts to apply physicochemical concepts to the behaviour of organic compounds in natural waters.

The author says that sometimes he has used "a clumsily detailed algebraic and numerical development" but this will be considered an advantage by many students trying to make the transition from description to a more rigorous approach. They will be assisted also by the frequency with which the text turns to the processes as they operate in nature, topical matters such as photochemistry and hydrothermal processes at ridge-spreading centres being included.

The material covered is inevitably similar to that dealt with in the well-established text by Stumm and Morgan, also published by Wiley, which went into a new edition in 1981. The present book has its own virtues, however, and should join *Aquatic Chemistry* on the desks of workers in the field. It is to be hoped that a cheaper edition may soon make it more accessible to students. □

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Yeasts and industry

Julius Marmur

Yeast Genetics: Fundamental and Applied Aspects.

Edited by J.F.T. Spencer, Dorothy M. Spencer and A.R.W. Smith.

Springer-Verlag: 1984. Pp.533. DM148, \$57.50.

IN 1970, Dr G.R. Fink offered the advice that "biochemists as well as geneticists should use only S288C or 'isogenic' *Saccharomyces* strains in their investigations. Regrettably, this has not been the rule in the past". True, the principal yeast of industrial importance is *S. cerevisiae*. However, there are over 400 yeast strains, harbouring a vast resource of genes encoding enzymes that carry out a variety of chemical transformations. What is very much needed is the comparative genetic and biochemical characterization of some of the more versatile strains, making use of classical and modern genetics.

J.F.T. Spencer and his colleagues have assembled a book on the principle that "the methods of yeast genetics have many

things to offer the breeder of industrial yeast strains". Thus, there are chapters on topics ranging from gene conversion to the breeding of brewing strains. While some subjects, such as gene conversion (dealt with here by Fogel, Mortimer and Lusnak) are handled in exquisite detail, others, nucleic acid relatedness among yeasts (Kurtzman, Phaff and Meyer), for example, are covered in a broad-based manner, bringing together a great deal of information that is otherwise hard to come by.

In addition to those subjects already mentioned which are "concerned with the nuclear yeast genome", there are chapters on the yeast cell division cycle (Carter, Piggott and Walton), meiosis and sporulation (Dawes), and radiation sensitivity and repair (Game). Particularly good is Elizabeth Jones's wide-ranging contribution on the genetics and biochemistry of yeast proteases and their inhibitors.

Extrachromosomal genetic elements are covered in three chapters, two of them on mitochondria. One would have been sufficient; the longer one (Evans), while initially intimidating, makes informative reading. Another contribution deals with

yeast killer toxins (Mitchell and Bevens) encoded by duplex RNA. The extensive discussion of yeast mitochondria in the book could be justified by an as yet ill-defined role of these organelles in affecting flocculation, sporulation and germination as well as sugar fermentation.

The industrial aspects of yeast are covered in contributions dealing with protoplast fusion (Freeman and Peberdy), genetics of wine yeasts (Snow), flocculation (Johnson and Reader) and in an excellent article by Kielland-Brandt and co-workers on genetic approaches (principally their own) to brewing yeast improvement. These chapters reiterate the message that the genetic analysis of industrial yeasts suffers from their poor mating ability, poor sporulation and poor spore viability. Snow's article briefly introduces an approach that is sure to be exploited, namely, the introduction of a heterologous gene (in this case the bacterial *Lactobacillus* gene encoding the malolactate enzyme) into yeast to modify or increase alcoholic fermentation. To this end the recent introduction of bacterial genes into yeast is notable, for example the gene from *Escherichia coli* coding for xylose isomerase which provides an important step in the hydrolysis of the plentiful supply of xylans.

Finally, the volume includes a chapter by Rank and Robertson on the composition of yeast plasma membranes (somewhat out of place in this book) and a broad review of yeast carbohydrate metabolism by Steward and Russell. What is missing is a chapter on recombinant DNA technology and sections with more information on excretion of extracellular enzymes and proteins.

The contributors are principally European with a sprinkling from North America. References are mainly up to 1981, with a few reaching into 1982. Although it is apparent that the genetics of industrial yeasts is just beginning, the chapters dealing with them do bring us up to date on what is known, often reviewing literature that is obscure to most molecular geneticists. And who knows: maybe some industrial yeast will become the S288C of the fermentation industry. □

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Second editions

- Volume II, *Animals*, of T.W. Goodwin's *The Biochemistry of the Carotenoids*. (Publisher is Chapman & Hall/Methuen; price is £25, \$50.) The second edition of Vol. 1, *Plants*, was reviewed in *Nature* 293, 494; 1984.
- *Macromolecules*, Vol. 1 *Structure and Properties* and Vol. 2 *Synthesis, Materials, and Technology*, by Hans-Georg Elias. (Publisher is Plenum; prices are Vol. 1 \$65, £50, Vol. 2 \$95, £73.15.) The new English-language edition is a translation of the German fourth edition. The original English edition was reviewed in *Nature* 270, 546; 1977.

Junior prometheans

David J. Rose

The Man-Made Sun: The Quest for Fusion Power.

By T.A. Heppenheimer.

Little, Brown: 1984. Pp.347. \$19.95.

In King Arthur's legendary domain, the Knights of the Round Table slew dragons and rescued fair maidens; only the pure of heart could hope to glimpse the Holy Grail. Heppenheimer likens the quest for controlled fusion to such an enterprise, but one pursued by more fallible human beings. In a gossipy book, he takes us through many activities and events, mainly in the United States, telling his story as a sort of passion play, even to the extent of including a list of 42 dramatis personae. It is an unbalanced work, divided into 12 relatively separate acts which are often named imaginatively ("Plasmas on the Potomac") but sometimes not so neatly ("Arms and the Man"). This is a nuisance for the reader, because each chapter has no breaks or subheadings of any kind, and nothing much in the way of a summary. An index is included, but no table of contents. Thus it is a hard book to read seriously, despite the conversational style.

Behind the apparent and sometimes real clash of personalities described by Heppenheimer, there were fundamental differences about how controlled fusion research should be done. Consider the course of events in Washington between 1980 and 1982 ("The Face Off"), leading to the resignation of Edwin Kintner, Director of the Office of Magnetic Fusion Energy, which was apparently triggered by the Office of Management and Budget's insistence that \$42 million — about 10% of the annual budget — should be shifted from new construction to operations. Although he fails to dig out all of the details, Heppenheimer gets the story mostly right — that the background issue was whether the time had come for proceeding more rapidly with larger test devices (Kintner's view), or whether then-current programmes should be better supported, to lay a stronger foundation for whatever might come (OMB's view).

A passionate argument lay behind this affair, which Heppenheimer did not fully uncover in his discussions with any of his 57 interviewees. It was this: what constitutes appropriate science and engineering in the daunting task of confining a nuclear fire with purely ethereal bonds, and getting cheap power out of it. Programmes and budgets refer to milestones, but the only accurate ones are on the road behind; no one knows how many lie ahead or where the end will be.

The search for fusion brought the whole glorious field of plasma physics to its maturity, but all of that science does not necessarily carry us into the world of

application. The research devices of today, marvels of engineering in their own right, may not be practicable when scaled up: can the grams of tritium fuel be regenerated and recovered from tons of (probably solid) moderator? Will the vast vacuum wall, thinner in proportion than an egg-shell, survive under neutron irradiation more intense than that in the core of a fission reactor, without leaks, for years? The structure will be radioactive and require remote maintenance more complex than do fission reactors. Other engineering difficulties exist, just as severe. At the end of it all, are fusion reactors likely to be made at a competitive price?

A characteristic of the whole fusion programme has been its dominance by the plasma physicists. But the quest for fusion power involves much more than science alone, and both workers and patrons came to expect too much, too easily, too soon. Robert Hirsch, Director of US Magnetic Fusion from 1972 to 1976 (to whom Heppenheimer devotes an entire chapter), managed to increase the budget from \$20–25 million to twenty times that amount, matching the scale of work to the task much better than before. But Hirsch left an unwelcome legacy — time-tables for the development of fusion reactors that presumed a state of scientific and engineering knowledge that just did not exist.

Kintner's agony had its roots in much the same sort of optimism. He advocated a series of ever larger experiments, as engineering test-beds. There were — and are — serious differences of opinion about whether that was the best way to proceed. Some engineering wisdom comes from such billion-dollar attempts, but does it come too narrowly, at too great a price? Other problems need working out more generally, and the answers are essential in helping us to judge the feasibility of fusion before resources become committed to a single giant device.

Heppenheimer devotes two chapters to laser fusion, starting with the late Keeve (Kip) M. Siegel, founder of KMS Fusion, and his chief scientific associate, Keith Brueckner. In the laser approach, a sub-millimetre pellet containing a deuterium-tritium mixture is subjected to a huge laser pulse that blows off the outer layers of the pellet, compresses it and heats it to fusion temperature. That scheme is related to how an H-bomb works. The somewhat delicate point arose whether Brueckner, who had some access to the technology, was using government-financed classified research for private commercial advantage. Apart from covering such tittle-tattle, Heppenheimer discusses this whole approach to fusion optimistically, an attitude that might find its explanation in the list of enthusiasts he interviewed.

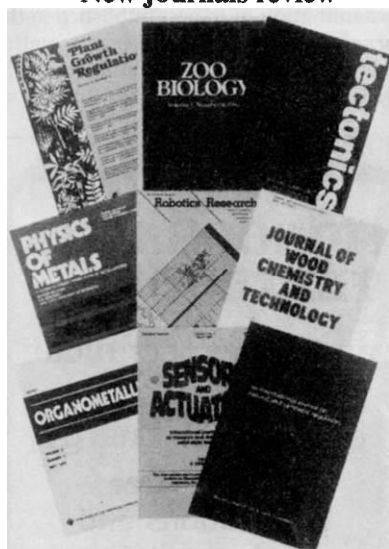
This kind of star-wars view of fusion energy (and perhaps of fusion research in general) seems to me to lose sight of the original goal: to provide a rather mundane

but essential service — energy — in as simple ways as are consistent with economic, environmental and social goals. Continuing experimental and analytical studies of laser fusion seem to be raising the technological ante for success faster than budgets, technology, and perhaps even science, can cope.

As to the characters and events themselves, Heppenheimer sometimes tells us too much — nine pages on a fight about whether an advance in achieved plasma energy was to be publicized, for instance. Are we really so interested in the appearance of the last part of Sir Galahad's horse to leave the stable? But in his last chapter, and occasionally elsewhere, Heppenheimer catches the spirit that inspired the pioneers in this enterprise. They were among the most brilliant of scientists, both in the United States and elsewhere, and did what they did because it was, in some deep way, a good thing for society to do. Some of that spirit still exists; without it, the quest will surely fail. □

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New journals review



On 27 September *Nature* will publish the fourth annual review supplement devoted to science journals.

Criteria for inclusion of a journal in the 1984 issue are that:

- (i) the first number appeared, or the journal was retitled, *between June 1982 and May 1983* (the second cut-off date allows at least three issues of a journal to have been published, the minimum number on which a reasonable judgement can be based);
- (ii) it is published at least three times a year;
- (iii) the main language used is English.

Publishers and learned societies are invited to send *four different issues of each suitable periodical, including the first and most recent numbers* (if from outside the United Kingdom, by air mail) to: The Review Editor, *Nature*, 4 Little Essex Street, London WC2R 3LF, England. Subscription details, if possible for both 1984 and 1985, should be included.

A different sort of Darwinism

J. H. Brooke

Darwin and the Spirit of Man.

By Alister Hardy.

Collins: 1984. Pp.245. £9.95.

SIR Alister Hardy is prominent among those who have argued for "Darwinism with a difference". Whilst acknowledging the creative powers of natural selection, he enshrines innovative behaviour as a distinctive selective force. His argument is that a learnt and beneficial habit can be instrumental in structural selection by favouring *subsequent* genetic change which might reinforce the behavioural novelty. In the approved words of R.F. Ewer: "behaviour will tend to be always one jump ahead of structure and so play a decisive role in the evolutionary process".

From such a possibility, Sir Alister advances the claim that behavioural selection has been one of the "major factors" in the evolution of birds and mammals. His sights are set on what he considers an "intellectual scandal" — the notion that Darwinian evolution can only be construed in materialist and reductionist terms. Using results from his Religious Experience Research Unit, his aim is to present an alternative synthesis which does justice both to the integrity of biological explanation and the reality of religious experience. So urgent a purpose in so distinguished a figure deserves serious consideration.

Informal in style, almost evangelistic in tone, the exposition strives for intellectual unity in a manner which strikes one as more impressionistic than formally rigorous. Even non-specialists may be perplexed by the way Sir Alister structures his alternatives. The reader is asked to choose between a chance mutation or a new habit as the instigator of adaptive change. Sir Alister's heart is with the latter, elevating the conscious, explorative and perceptive aspects of animal behaviour. But may not the choice itself be misleading? If a bird learns a new trick, even how to raid a milk-bottle, the capacity to learn was presumably already there in the gene-complex, having been encouraged by past selection. And if the new behaviour continued to be advantageous, it would presumably be favoured, again by the normal process of selection. Conversely, a mechanism whereby genetic change *subsequently* reinforced a behavioural innovation might invite the objection that it could destroy a useful flexibility of response. The new habit, if genetically and structurally "fixed", might sacrifice versatility for a precarious short-term gain.

A constrictive choice is also presented in the treatment of consciousness: either an extreme reductionism, in which conscious-

ness is epiphenomenal; or a form of psychophysical dualism, affirmed on eminent authority but largely assumed by Sir Alister rather than specifically argued for. Excluded by this dichotomy would be those monistic accounts which treat consciousness as an "emergent" property, associated with a particular degree of material organization, but neither reducible to it nor the product of an elusive spiritual element. It is Sir Alister's view that the spiritual element is not, after all, elusive. It exists in all mankind and may be discerned in numerous reports describing a



Sir Alister Hardy — "Whilst acknowledging the creative powers of natural selection, he enshrines innovative behaviour as a distinctive selective force".

"feeling of receiving help from a power, from Divinity, beyond the self".

The perplexity here is that whilst the author offers a sensitive classification of reported experiences, it is not shown how, from reports alone, one can establish anything further than the point that such feelings of dependence are common. The counterpoise is still that which weighed so heavily with the young Charles Darwin as he studied the natives of Tierra del Fuego: such feelings were apparently not shared by, and therefore not essential to, all humanity. The further suggestion that this sense of drawing upon a power outside the self might have become an energizing force in human evolution, a "something that gave more courage than [men] had ever had before", is not without difficulty. Might that sense of dependence not just as easily encourage passivity as activity? Alternatively, supposing it had gradually been reinforced by its survival value, would that really persuade us that man's spiritual nature had any transcendental significance? But whatever one's response to Sir Alister's confessedly personal view, it is stimulating to engage his own burning questions. □

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Automation is just beginning

The laboratory application of computers is being extended rapidly, but there is some way to go before the penetration of these machines is as complete as, say, that of the Bunsen burner.

A FEW years from now, the heading on these pages will be entirely inappropriate. The idea that the use of computers in laboratories is sufficiently novel to be remarked upon will have ceased entirely to be noteworthy, just as it would now seem strange to assemble a few pages of this journal under the heading "oscilloscopes in the laboratory". So charitable readers are invited to regard the pages that follow as a reminder that the present is a transitional period, one in which it is not yet second nature for experimentalists and theorists alike to use all the tools now potentially at their disposal.

It is also worth remarking that the present hectic period during which the laboratory computer is being made familiar has its roots in a period which long predates the development of electronic computing machinery. One of the obvious illustrations of this truth is in the development early this century by C.T.R. Wilson of the cloud chamber, first for stimulating processes occurring in the atmosphere, later for the recording of cosmic ray particles by the trail of droplets produced in a supersaturated vapour. The first studies of cosmic rays were necessarily made with cloud chambers whose expansion (to give supersaturation) was carried out at random. But the technique became productive only when, in the 1920s, cloud chambers could be made to record events only when the chance of something interesting being observed was high.

The (Geiger) counter-controlled cloud chambers of those days are forerunners of the computer-controlled experimental rigs that are now commonplace in many important ways, but not least because they stimulated the development (with thermionic valves or tubes) of the logic circuits now embodied by the thousands in microprocessors. Over half a century, the practice of particle physics has been inextricably bound up with the development of electronic techniques for making sure that observations are made only when it is profitable to do so. And while the techniques have now spread to a host of other fields in which the objective is to record transitory phenomena, accelerator physics remains the citadel of the selfconscious practice of selecting from a mass of naturally arising happenings only those about which observational data are likely to be useful.

Computer-controlled experiment has the virtue of economizing in effort in the

way most likely to be productive — by the avoidance of unwanted data. This is the spirit in which, in many biological applications (and more generally), it is possible to record data only for as long as necessary to provide some predetermined degree of statistical certainty. The prerequisite of such procedures is that there should be a computing facility "on-line" with the data-gathering equipment, carrying out an analysis as the experiments proceed. Again, inevitably, the accelerator people have blazed a trail which everybody will soon be compelled to follow. For the collection of needless data is not merely inelegant but an encumbrance, a distraction. Even now, however, the point is not sufficiently forcefully made in teaching laboratories.

The notion that computers in one form or another have an important part to play in the collection ("logging") and analysis of data is, by comparison, more readily accepted. What seems to be less widely appreciated is that there are already a great many kinds of investigations now commonly undertaken that would be beyond the scope (and stamina) of people without the assistance of computing machinery. Particle physics has again been the pathfinder in this trend, with its techniques for the recording of tracks (or points along the tracks) left by particles in detectors of various kinds, and for their subsequent automatic analysis.

Inevitably, this practice has spread like wildfire. Deep seismic refraction, both in the exploration for petroleum and in geophysical investigations, generates such masses of data that only computer recording and analysis is feasible. The identification as well as the measurement of stellar and galactic objects on photographic plates collected by astronomers is similarly a way of carrying out tasks that would otherwise be impracticable. And even where the quantities of data are not nearly as overwhelming, there remains the possibility that they will not be fully interpreted because the investigator has not the stomach for the task or, more charitably, takes the view that his priorities lie elsewhere. Yet even in this uncontroversial field, where the value and the objectivity of automatic data analysis is generally accepted, teaching laboratories are not nearly as well equipped as they might be.

The application of computers as means of retrieving data is, by comparison, commonly undervalued. Many people

have found the widely available bibliographical databases less than a substitute for the professional literature. And it is true that many of these systems are needlessly wooden and inflexible. There may be more promise in the schemes (some of which are referred to in the pages that follow) that assist people to construct their own individual databases, for mnemonics are almost certain to be more "friendly" (in the computer jargon) than substitutes for one's memory.

The use of databases simply for the recognition of meaningful portions of the nucleotide sequences of DNA which have become a common feature of the literature is perhaps the most telling illustration. The plain truth is that it is almost conceptually impossible for a person to compare the sequence of some nucleotide sequence he has constructed with a library of known gene sequences so as to pick out those which most closely resemble each other. Not so long ago, an author of an article submitted for publication was much chagrined that a referee with access to a better computer program was able to spot a similarity that had previously gone unrecognized. But this is only one set of circumstances in many where the full power of computer retrieval remains to be recognized. Rivalry between laboratories will no doubt bring other cases to light.

But none of this, it will be remarked, bears on what must seem the most obvious application of computers in the laboratory — the accomplishment of mathematical tasks that would otherwise be beyond the scope of ordinary people. The use of powerful computers for the simulation of, say, the evolution of weather systems requires no special justification, but the huge installations by which these tasks are accomplished are (and are likely to remain) the exceptions, not the rule.

More interesting are the applications of computer programs to ordinary tedious tasks of algebraic analysis, still regarded as a kind of frivolity or luxury, and to fields in which the underlying characteristics of a mathematical process would not otherwise be apparent. Who would at this stage claim that the importance of chaotic (or "deterministically random") phenomena would have been widely recognized without the computer simulations of the process that are now commonplace? Or that the study of fractal systems could have been accomplished analytically if only mathematicians were less idle? □

Computers in books

Microcomputing in biology

A.N. Barrett

GIVEN the shortage of suitable software for analysing biological data by microcomputer, the publication of books containing methods and programs for that purpose is generally all to the good.

One recent book of this type is *Statistics and Numerical Methods in BASIC for Biologists*, written by J.D. Lee and T.D. Lee and published by Van Nostrand Reinhold (hbk £14.95, \$32.50; pbk £7.75). The authors include methods for determining means and standard deviations, chi-squared tests, various correlation coefficients, linear regression and curve-fitting. There are also two chapters on the biological applications of statistics: one on assays, the other on linear regression techniques applied to bacterial growth data.

Accompanying the methods are some 20 programs, written in elementary BASIC, which are highly suitable for laboratory use. The programs are well structured and incorporate numerous checks against incorrect or pathological data. Documentation is provided with the programs, enabling the user to follow easily the program structures, and sample data is available for users to test the programs on their own computer. One price of the robustness of the programs, however, is that they are considerably larger than similar programs performing the same calculations. And to ease typing problems, it would be useful if the programs were available on a floppy disk for the Apple and BBC microcomputers.

A book with similar statistical content is *BASIC Microcomputing and Biostatistics* by Donald W. Rogers (Humana; \$39.50 (US), \$49.50 (elsewhere)). The aim of the book is to teach essential programming skills, using BASIC, and to provide a range of statistical and mathematical techniques for the analysis of data in the physical and life sciences (including medicine).

The book starts with a rather brief discussion of computing and BASIC, with three simple programs illustrating certain BASIC functions. The next seven chapters deal with a variety of standard statistical techniques and also provide simple programs to illustrate the application of the techniques to limited data sets. The statistical methods introduced cover the understanding of experimental errors and the computation of means and averages, and the determination of probability distributions together with the use of the Poisson and Normal distributions. A chapter is also included on the application of the chi-squared test and Student's *t*-test. Finally, least squares are explained, as well as the fitting of nonlinear curves and the solution to simultaneous equations.

From the point of view of the laboratory scientist, one of the limitations of Rogers's book is that many of the programs are unsuitable in their present form for analysis of experimental data. The curve-fitting program, for example, only extends to a second degree curve and several of the earlier programs are restricted to the determination of properties of groups of five numbers. The principles used do not easily lend themselves to extension of the programs to handling large data sets and as such are of limited value except for teaching. In this context, however, the book achieves many of its stated aims; a lot of examples are provided and the programs are well supported by background information.

In contrast to the preceding two books, James D. Spain's *BASIC Microcomputer Models in Biology* (Addison-Wesley; \$34.95, £22.10) contains few programs and concentrates on providing flowcharts to accompany the methods from which the readers themselves can develop programs. Several useful programs in BASIC are available, however — for curve-fitting, polynomial fitting, normal distributions, queuing simulation and graph plotting — either as listings contained in an appendix or on a floppy disk (price \$50). The programs run on either the Apple II or TRS-80 computers. The appendix also includes an introduction to programming in BASIC.

The main text is divided into three parts. The first deals with simple equations that model single biological systems or system components such as exponential growth and enzyme saturation. The second is concerned with deterministic models of multicomponent systems and includes, for example, chapters on biochemical reaction kinetics, dynamics of homogeneous populations of organisms, models of microbial growth and simulations of population genetics. The final part deals with the effects of random processes on biological systems, with special attention being paid to sampling processes, random walks and queuing.

As a means of introducing both students and practitioners of biology to theoretical methods and their applications, Spain's book fulfils a useful purpose. Its structure will appeal in particular to those with sufficient programming skills to translate the flowcharts into working programs.

Finally, *Microcomputers and Physiological Simulation* by James E. Randall (Addison-Wesley; \$27.95, £14.50) is a soft-back aimed at providing an introduction to microcomputers and their use in the mathematical simulation of physiological processes.

The first five of the 14 chapters present a discussion on both hardware and software.

and include accounts of microcomputer components, and operating system and programming languages, together with graphics facilities for the TRS-80 and the Apple II. Each chapter concludes with a list of addresses of manufacturers of both hardware and software, where appropriate, and with references to relevant journals.

For a book dealing with techniques of mathematical simulation, surprisingly little background mathematics is included. Instead, the author concentrates on providing BASIC (Apple) programs encapsulating the relevant mathematical equations necessary for the simulations and refers the reader elsewhere for the supporting theory. The complexity of the programs varies with the particular application (axon potentials, cardiac action potentials, distortion of physiological waveforms and the glucose tolerance test are among those covered).

Randall's book clearly demonstrates that a variety of physiological processes can be successfully simulated on the microcomputer. It should be of interest to advanced students and physiologists with interests in modelling. □

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Computers and genes

E.G. Richards

The Applications of Computers to Research on Nucleic Acids II, Parts 1 and 2.

Edited by D. Söll and R.J. Roberts.
IRL Press: 1984. Pp.856. Pbk £30, \$60.
Time Warps, String Edits and Macromolecules: The Theory and Practice of Sequence Comparison.
Edited by D. Sankoff and J.B. Kruskal.
Addison-Wesley: 1984. Pp. 382.
\$28.95, £24.60.

THERE are over one-and-a-half million bases in the latest edition of the EMBL nucleotide sequence library and somewhat more scattered through the literature. If printed, they would comfortably fill the two volumes edited by Söll and Roberts and totally bemuse anyone who opened the pages of such a book. It is then no small wonder that the writing of computer programs to aid the construction and digestion of nucleic acid sequences is a growth industry. The two volumes were originally published as Volume 12, Number 1 of the journal *Nucleic Acids Research* in January of this year. A similar work, half the size, was published in January 1982, giving us a doubling time of two years.

The three parts of the latest volumes are devoted to main-frame computers. mini-

computers and microcomputers. The 79 papers describe programs and systems which range from the grandiose to the trivial. Several of the papers describe large menu-driven systems for processing sequences, including printing out sequences in various formats, translating them into amino acid sequences and searching for restriction sites to mention but a few of the options. Other contributions are devoted to more specialized tasks, such as working out restriction maps, ascertaining the homology between sequences, determining the most stable base-pairing scheme in an RNA chain, calculating the molecular size of fragments observed in polyacrylamide gels and even designing your experiment for you. There is only one paper devoted to the graphic display of three-dimensional structures; this is notable for being implemented on a microcomputer.

Molecular biologists engaged in producing nucleic acid sequences or cogitating on their significance are likely to find something of interest in the two books. It may be that the author will send them a listing when asked (some offer so to do, others are silent on this point), that the program is written in a language and dialect familiar to their local computer, and that appropriate hardware is available. If all this is so, such researchers may be saved hours of work reinventing the wheel. But it is unclear whether the programs themselves have been verified in any way, and hard to tell how robust they are against misuse or how easy they are to use.

Most of the programs concerned with homology and structure prediction are based on the dynamic programming method discovered, apparently simultaneously, by several workers in the 1970s. This method, based on an argument by induction for ascertaining the minimum number of insertions, deletions or changes required to transform one sequence into another, is well described in *Time Warps, String Edits and Macromolecules*.

In this book, various contributors explain how almost identical problems arise in several disparate fields, including speech recognition. Here the pressure wave corresponding to the utterance of a word is sampled at discrete times and a resulting vector compared with that of a standard word. Since people vary in the rate at which they speak, the sampling rate must be varied in some way; that is, time must be warped.

Much of Sankoff and Kruskal's volume should be accessible to molecular biologists interested in the intricacies of sequence comparisons and similar problems, and should serve a useful role in acquainting them with the state of play in this area. Other parts are more mathematical and thus more suitable for the computer specialist. □

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How to crunch your numbers

Gordon C.K. Roberts

Biodata Handling with Microcomputers.

By R.B. Barlow.

Elsevier Biosoft, 68 Hills Road, Cambridge CB2 1LA, UK: 1983. Pp.261. £18. \$30. Disks £30, \$50.

EVEN the least expensive "home computers" are capable of carrying out all the statistical analysis that most biologists will require — and usually before the answer can be obtained from the computer centre. The main obstacle to practical realization of this is, of course, the limited availability of appropriate programs. Behind the somewhat mysterious title (what is "biodata"?), this book goes a considerable way towards meeting the need. It contains some 50 programs which cover most simple statistical procedures, from standard deviations and significance tests through analysis of variance to linear and non-linear (iterative) regression.

The book contains listings of the programs (in Commodore PET BASIC), repro-

duced from a dot matrix printer, while the text is reproduced from typescript — both are entirely legible, although the multi-statement lines and lack of indenting make the programs harder to follow than they need be. White space is prominent, since many pages are under two-thirds full, and there are several rather un-funny cartoons.

Each program is accompanied by a brief but generally adequate commentary describing what is going on, and in many cases also by an even briefer outline of the underlying mathematics. Beginners may need a statistical text to amplify some of the explanations if, as the author hopes, they wish not only to use this book as a "toolkit" but also to understand how the tools work. In addition each program has a sample output, the examples being drawn primarily from pharmacology.

The programs cover clearly the most widely used statistical procedures, and are available on disk for Commodore PET, Apple and Commodore 64 computers. The combination of disk and book certainly adds up to a useful elementary statistical package. □

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Pascal in practice

Graham H. Kirby

Pascal Applications for the Sciences: A Self-Teaching Guide.

By Richard E. Crandall.

Wiley: 1984. Pp.246. Pbk £13.90, \$16.95.

THE number of textbooks which deal with computer programming in Pascal is very large and still increasing. So it is refreshing to find that Richard Crandall's book concentrates on *applying* the language. The author's aim was to provide a text which would enable students of mathematics, physics, chemistry and biology to learn scientific programming in Pascal. Applications in each of these specific subjects follow a general introduction to simple numerical programming.

The book is a self-teaching guide, consisting of text, exercises, answers, more text and so on. Some exercises involve modification of the many programs presented in the text whilst others are distinctly ambitious. Many answers are short comments and hints rather than code. One of the good features is the emphasis on graphics to display computed results, rather than on the printing of tables of

numbers, and a microcomputer with graphics facilities and Pascal would provide the ideal basis from which to benefit from the book. Also commendable is the provision of source code for libraries of Pascal procedures covering graphics, matrix handling, statistics and specialized mathematical functions. Of course, the graphics library is hardware specific (for Tektronix 4012 terminals).

A book such as this, modestly-priced and devoted to applications, has insufficient space to introduce the language itself. Readers will require either a prior knowledge or an introductory textbook on Pascal programming. Without one or the other, the benefits of Pascal will not be appreciated since Crandall has concentrated entirely on the mathematical modelling type of application using simple numerical and statistical techniques. This is a pity because the full power of Pascal is not demonstrated — I noted very few occurrences of the type *char*, only one of records (for complex numbers) and of the case statement, and none of pointer types. The programs could be re-written in FORTRAN or BASIC with few changes.

Science students will find this book helpful in learning to write simple Pascal programs for applications in their subject area. I hope they go on to learn more of the facilities in Pascal; many scientific applications do require data structures other than arrays and types other than integer and real. □

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● Reston/Prentice-Hall have just published *Scientific Pascal* by Harley Flanders, a book written specifically to teach practical programming to scientists and students of science. Price is hbk \$29.95, £28.45; pbk \$21.95, £20.85.

Sorts of references

K. V. Roberts

PAPERBASE: A Computerized Personal Database for Scientists.

By Anthony Durham.

Wight Scientific, 44 Roan St, London SE10 9JT. Booklet, pp.31, £25, \$50 (airmail postage included) for institutional use; £10, \$20 for personal use. Diskettes available for various computers, £50, \$100 (booklet included).

PAPERBASE is a BASIC package for constructing and interrogating a database of references. It normally uses a micro-computer with diskette drives, screen, keyboard, printer, BASIC interpreter and word-processing program, although it can run on larger or smaller systems. I tested it on an IBM PC-XT and found the increase of speed provided by a Winchester disk and the BASIC compiler to be an advantage, although it is still a very useful system without these facilities.

References and keywords are input using program STORE: it is important to get the format correct, but this is not difficult and one can edit the ASCII output file later (for example to add new keywords). ORDER sorts alphabetically by first authors and an on-line search on logical combinations of expressions is carried out by SEARCH, producing a file containing those references that meet the given criteria. References are converted to the format required by a particular journal by LAYOUT, using word-processing facilities such as bold-face and underlining where appropriate. The user may need to develop his own format code since journal practice differs so widely, but examples are given and it should not take long to master the rules. Finally, HUNTER scans through an ASCII file — for example a typescript produced on a word-processor — to report the references that it finds.

The BASIC program may have to be modified before use, depending on the computer. For the IBM PC no changes were necessary and PAPERBASE ran straightaway. However, the code is well documented in the booklet, and advice is given on how to make changes should these be needed. Users may also wish to extend the programs to meet additional requirements or to build them into larger systems; I found it helpful to modify SEARCH so that it did not distinguish between upper and lower case, and this was readily done with a few BASIC statements although it somewhat slowed down the search.

Altogether PAPERBASE is a well-engineered piece of software. It should find wide use throughout the scientific community. □

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New computer products

By using the Reader Service Card, details of twenty-one new computer based products can be obtained.

- The Series 7000 professional computers from Perkin-Elmer are advanced micro-computer data processing systems specifically for laboratory applications. Two models are available: the PE 7500 and PE 7300, capable of both analytical and management functions. In an integrated workstation, a Series 7000 computer is complete with a detachable keyboard, colour or monochrome graphics display, Winchester and floppy disk drives. The electronics module contains a Motorola 68000 16-bit microprocessor which has 32-bit internal data pathways and can address up to 16 megabytes of random access memory (RAM). A Series 7000 computer contains 640 kilobytes of RAM as standard. The internal memory can be expanded by one megabyte by the addition of an internal expansion board. Perkin-Elmer produces applications software for instrument control, data collection, data storage and graphical manipulation for infrared, ultraviolet/visible, fluorescence and atomic absorption spectroscopy, gas and liquid chromatography and thermal analysis. Users can write their own programs in BASIC and FORTRAN.

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- Beckman Instruments' SYNLIB is a computer software system for the creation, maintenance and search of chemical reaction data files. The SYNLIB program has been designed specifically for synthetic chemists involved in the manufacture of organic chemistry products, pharmaceuticals and chemical specialty products. The SYNLIB system is distributed with a database of over 12,000 chemical reactions abstracted from the scientific literature. A continuing database updating service is available, and users may easily establish, maintain and search their own chemical databases containing chemistries which are of local interest.

Circle No. 101 on Reader Service Card.

- TUTSIM is a program for the simulation of contaminous dynamic systems, and is now available in a short form which is suitable for evaluation, demonstration and instructional study. TUTSIM is available for IBM XT/PC, and PC jr, Apple II and some CP/M machines. The TUTSIM program was created by the Twente University of Technology in the Netherlands. It is a block diagram orientated program which produces both numeric and graphics output. Disciplines to which TUTSIM is applicable include physiological and biological model studies, thermodynamics and fluid dynamics and econometrics and sociological systems. TUTSIM is available in the United States from the Applied i company of the Palo Alto, California.

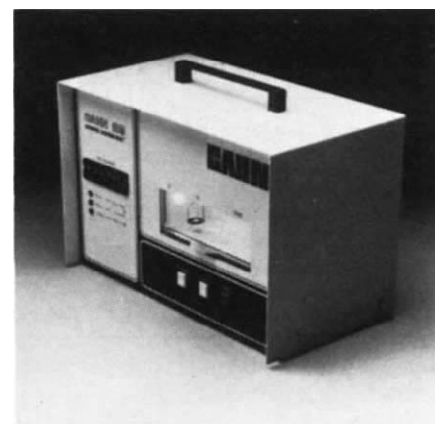
Circle No. 102 on Reader Service Card.

- The Cahn Model 28 microbalance and C-29 ultra-microbalance now include as standard equipment the RS-232 interface for printers, computers and other peripheral equipment. Weighing to a precision of 0.1 microgram, Cahn Series 20 microbalances offer automatic calibration, wide dynamic ranges, remote weighting in glove boxes, hot boxes or environmental chambers. Operation is simple, as no internal weights or special set-ups procedures are required. Both the Model 28 and 29 balances are portable. The micro-processor controlled microbalances have capacities of 3.5 grams.

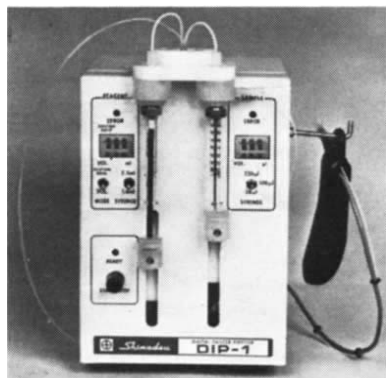
Circle No. 103 on Reader Service Card.

- The LABSAM/3350 is a Hewlett-Packard software package designed to manage and organize the flow of samples through the laboratory. LABSAM's primary functions include sample log-in, result acquisition, validation and reporting. LABSAM is designed for use with the HP 3357 Laboratory Automation System which features: disk storage up to 1 gigabyte; BASIC, FORTRAN and PASCAL languages; a wide range of terminals and printers; and a large selection of graphics devices.

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Perkin Elmer's professional computer (top) and the Cahn 29 balance



The Shimadzu DIP-1 diluter/pipetter

● A new data and chromatography control system allows chromatographers to set up HPLC methods in plain English and to control all system functions from one keyboard. The Waters 840 control station incorporates the Digital Professional 350 computer with Waters Expert chromatography software. The separation can be followed while it happens with real-time plotting on the screen, or the system can gather raw data from an analysis while the screen is used to perform other tasks. A high-speed, high capacity Winchester hard disk stores methods and up to one month of continuous raw data from up to four detectors. Dual floppy disks provide long-term storage of methods and data and allow easy recall for reprocessing or comparative analysis. In addition, integration methods can be changed in seconds to optimize data reduction, or the Waters SCANNER routine can be used to reprocess individual peaks at the touch of a button.

Circle No. 105 on Reader Service Card.

● Technical Database Services (TDS) of New York is offering a new search and retrieval service for the Log P Database. Subscribers at remote computer terminals can search over 25,000 records containing partition coefficients and related information for over 12,000 organic compounds in approximately 300 solvents. This compilation of Log P data results from a cooperation between the Pomona College Medical Chemistry Project and scientists throughout the world. The database, now in its fourteenth year, is updated semi-annually and increases by approximately 2,500 records each year.

Circle No. 106 on Reader Service Card.

● The new D470 titration kit from Radiometer is a complete package connecting Radiometer titration equipment to a Hewlett-Packard HP-85 personal computer. The fully documented standard tape is pre-programmed to perform automatic titrations with the simplest possible operation; only 4 soft keys have to be operated for routine titration. Equivalence points are automatically detected as the inflection points of the titration curve. The titration results and curve are displayed on the screen of the HP-85 and can be plotted by the built-in printer/plotter.

Circle No. 107 on Reader Service Card.

● The Shimadzu DIP-1 is a simple-to use computer controlled diluter/pipetter. Just set the dilution ratio on the digital dial, selectable in 18 steps of 2 to 100, and it will accurately dilute the sample at that ratio on dispensing. The unit can also be used for dispensing, sampling, and diluting-dispensing. When only a reagent is to be dispensed, if the set volume is larger than the full stroke volume of the syringe it will automatically repeat to provide the desired volume up to 9.99ml. When an extremely small sample is to be taken (5 to 25 μ l) the volume required is set on the digital dial, and the tip of the nozzle placed in the sample. At one press of the "start" button the volume of the sample will be drawn up, and when pressed again the sample will be expelled into a container. When used for diluting-dispensing, the volumes of reagent and sample are set, and all the rest is performed at a press of the "start" button.

Circle No. 108 on Reader Service Card.

● AUTOSCAN, from Spectra-Physics is a BASIC program on a PROM chip for automatic stop-flow peak scanning, absorbance ratio determinations, and time-programmable wavelength changes. The program is intended for use with the SP8440 UV/VIS variable wavelength detector in conjunction with Spectra-Physics HPLC systems and the SP4200 computing integrator. In addition to reporting absorbance ratios and plotting of spectra, AUTOSCAN allows for completely unattended operation since the HPLC pump start/stop functions are integrated into the program software. The chip plugs directly into the memory board of the SP4200 computing integrator and is the latest addition to Spectra-Physics Special Purpose Software Programs (SP)³ series. This firmware-based system eliminates the need for loading programs from auxiliary storage devices such as disk or magnetic tape.

Circle No. 109 on Reader Service Card.

● A Thermex interface converts Apple II Plus or Apple IIe computer into an automatic-printing 16 channel thermometer. The interface uses J, K or T thermocouples as sensor, and resolution in the biological temperature range is 0.1°C. At other temperature ranges an accuracy of 0.28% full-scale is achieved using individually calibrated thermocouples. A special calibration program is available, allowing the user to calibrate and linearize thermocouple probes in any desired range. Thermex, available from Columbus Instruments of Ohio, has built-in cold junction compensation. Biological probes for rectal, intravenous and implantable applications are available for animal use. A video monitor displays current temperatures in all channels. Data are also periodically printed and saved on disk at selected intervals. An optional data-transfer package allows information to be transferred from disk to files to a host computer via an RS-232 interface.

Circle No. 110 on Reader Service Card.



The Radiometer D470 (top), the AMICA titration system (centre) and a thermometer for use with Apple computers

● Routine photometric measurements, potentiometric titrations of all kinds and photometric titrations can be performed with the computer controlled AMICA system from Hamilton. The titrimetric methods used include acid-base titrations, redox titrations, titrations in non-aqueous media photometric titrations with or without indicator and complexometric titrations. Because of its modular design an AMICA system can be adapted to the individual needs of any analytical control laboratory, whether the need is a small number of samples and frequent change of method, on many samples for few models.

Circle No. 111 on Reader Service Card.

● ELISA-CALC, available from Comple-Software of Iowa City, is designed to transfer data from an automated ELISA plate reader to a personal computer. The data are then saved on floppy diskettes; each diskette will hold data from more than 500 plates. ELISA-CALC allows the user to enter a custom plate "map" defining the contents of each well on the ELISA plates; this map is saved on the diskette so that it can be used indefinitely. The computer displays graphically each standard curve, either as a semi-log or as a log-log plot. It then allows the user to pick the appropriate points to produce the best line to fit each curve. Then it calculates each unknown on the plate using the appropriate standard designated on the plate map. It allows the user to calculate ELISA data immediately after the plate has been read, spending two or three minutes rather than hours per plate. ELISA-CALC is available to run with automated ELISA readers sold by Bio-Tek Instruments, Inc., Dynatech Laboratories, Inc. and Flow Laboratories, Inc.

Circle No. 112 on Reader Service Card.



Biodata's Microlink interfacing system

● The CmC BUSSter RGA is a micro-processor-based interface designed to give RS-232 computers and terminals the ability to function as IEEE-488 controllers. Up to 15 individually addressed IEEE-488 devices can be connected through the BUSSter RGA to one RS-232 port. The BUSSter RGA serial port features include switch-selectable baud rates (300 to 19,200), parity and word length. It can be configured for talk and listen commands for any IEEE-488 address or secondary address from 0 to 31. Multiple devices can be addressed at the same time. The RGA is also compatible with service request, serial poll, parallel poll, remote/local, local lock-out, device clear, and device trigger interface functions.

Circle No. 113 on Reader Service Card.

● Coulter Electronics have now introduced into Britain the Micromeritics computer-controlled DigiSorb 2600 gas adsorption analyser. This completely automated physisorption instrument may be used with a wide variety of adsorbates to determine surface area, pore volume, pore size and pore area. Features include operator selectable parameters, automatic pressure transducer calibration, sample outgassing to 550°C and a large liquid nitrogen reservoir. Unattended operation is possible for up to 4 days. Automatic data and graphic presentations are printed for up to five samples.

Circle No. 114 on Reader Service Card.

● The Philips Automated Laboratory Management (PALM) system can tackle both sample analysis control and industrial process/quality assurance. A typical PALM installation might include automatic sample preparation machines with conveyor links to a laboratory or directly to an X-ray spectrometer. The PALM system is principally intended for use in conjunction with Philips equipment, but instrumentation other than Philips may be used. Results from various sources can be merged to give a complete analytical report with a minimum of delay, via terminals or printers in the laboratory or at remote locations.

Circle No. 115 on Reader Service Card.

● Integration of chromatography data can be achieved swiftly using Autovideo-gration software for the Apple IIe or II+ computer. The program, from Heyden Datasystems, can be used with thin layer chromatography as well as being applicable to many GC and LC needs, especially when fewer than 20 peaks are being measured. Raw data may be viewed on the screen with scale expansion. Baseline position is under user control and integration may be automatic, manual or peak by peak. Results are tabulated with percentage peak areas, and all data can be stored to disk for subsequent examination.

Circle No. 116 on Reader Service Card.

● Two new features for GenBank, the genetic sequence data bank from Bolt Beranek and Newman Inc. (BBN), make for improved accessibility. A new interface allows the user to access GenBank data via a short series of menus and dialogues, and access is now possible through Telenet, an internationally available communications network. This eliminates the need for dialling into the system via long-distance telephone lines. GenBank, developed by BBN and Los Alamos National Laboratory for the National Institute of General Medical Sciences of the National Institutes of Health, is a repository of all published nucleic acid sequences greater than 50 nucleotides in length, catalogued and annotated for sites of biological interest.

Circle No. 117 on Reader Service Card.

● The publishing company Elsevier has introduced a range of scientific software with source code listings. The programs now available are: REVALUE: a software package to calculate reference intervals from total hospital patients laboratory data (for HP 85, PDP 11/23, PDP 11/45, PDP 11/70, VAX 11/780); BALANCE: a program to compare two series of measurements (Apple, IBM PC); and INSTRUMENTUNE-UP: an experimental optimization program, which helps the user to improve the performance of common scientific laboratory instruments (Apple, IBM PC).

Circle No. 118 on Reader Service Card.

● Electroplan of Royston, Herts, specializes in interfacing analog and digital signals from scientific instrumentation and laboratory experiments into many types of desk-top microcomputers. Systems available include Biodata's Microlink, which consists of a card frame into which modules are slotted to build up a flexible interfacing unit. Microlink connects to computers using the standard IEEE-488 interface, and interface cards and programming software are available for use with Hewlett-Packard, IBM, BBC, Commodore CBM PET, ACT Sirius and Apple computers. Electroplan is also UK distributor for Tecmar Labmaster and Labtender, which are data acquisition products designed to plug directly into the IBM-PC and other compatible computers. These are multi-function cards, offering 16 analog inputs, a 24-line general purpose digital input/output interface, and 5 event counters and timers on one convenient plug-in.

Circle No. 119 on Reader Service Card.

● Beckman's products for laboratory information processing can be divided into three categories: data acquisition; data analysis; and sample, test and method and result management. Data acquisition includes intelligent instruments, RS-232 interfaces, standard information protocols and interfaces for other manufacturers' instruments. Data analysis includes application-specific software packages for such applications as tablet dissolution, kinetics, content uniformity and gel scanning analysis. Sample, test and method and result management includes large-scale systems for automating entire laboratories. Beckman systems interface directly to all laboratory instruments from all major manufacturers. Both instruments and accessories can be controlled. The systems are directed by laboratory-developed methods. MK 4 and MK 5 Digimetry instrument couplers provide for interfaces with analog instruments, as well as instruments without an RS-232 interface or standard information protocol. Data acquisition programs can run on personal computers, as well as large systems. Beckman's systems provide extensive data analysis for general chromatography, simulated distillation, gel permeation, continuous flow and potentiometric titration. For sample, test and method and result management, the database is determined by the laboratory and updated on-line. The systems provide a configurable operational sequence and configurable sample and test status requirements according to the laboratory's specifications. The systems feature long-term archiving and recall. Beckman systems can communicate with other CALS systems, personal computers and mainframe computers.

Circle No. 120 on Reader Service Card.

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.

Physics

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Biotechnology manpower in the UK

from Richard Pearson

The best brains will not remain in this country unless the best jobs are here too.

BIOTECHNOLOGY is one of the fast growing new technologies. It was born as late as the 1970s and is recognized everywhere as a key technology for the future. As well as the United States and Japan, the technology superpowers, France, West Germany, Switzerland and the United Kingdom have taken initiatives to develop their biotechnology, even though the industry has barely moved out of the fundamental research stage. Being a knowledge-based industry, the availability of skilled manpower is crucial to its future success. In the United States, the Office of Technology Assessment has assessed prospects for that country and also provided an international overview, and has highlighted the strengths and weaknesses of different countries' manpower (see *Nature* 307, 399; 1984). In the United Kingdom, the Science and Engineering Research Council (SERC) has been trying to ensure adequately trained manpower to support this emerging technology and has just published two reports prepared for it by the Institute of Manpower Studies (*Enabling Manpower for Biotechnology* and *The Biotechnology Brain Drain*).

There are now almost 2,000 professional specialist personnel active in novel biotechnology in the United Kingdom in addition to those engaged in traditional areas such as brewing. While a precise definition of a "biotechnologist" is inappropriate, most individuals being regarded as, say, microbiologists, biochemists or biochemical engineers, it is clear that, on the science side at least, few people would be regarded as professional biotechnologists without three or more years postgraduate experience; on the process engineering side, however, the entry threshold is more likely

to be a first rather than a higher degree.

The 2,000 specialists in the United Kingdom are employed in more than 100 organizations, some with just one or two, others with more than 50. One research laboratory employed over 100. The type of organization involved did not, however, indicate the actual employment level — two major pharmaceutical companies, for example, each employed fewer than 25 specialist biotechnologists, much of their basic research being contracted out to university-based research groups.

The commercial sector accounted for nearly half the employment at professional level, with research institutions taking about a third and the balance in higher education, although in the latter it was sometimes difficult to isolate applied biotechnologists from those focusing on more fundamental aspects of science (Fig.1). With the rapid growth of commercial companies spinning off from academic departments, consultancies and so on, the dividing line between the sectors is also becoming increasingly blurred.

With the exception of junior staff and chemical engineers, who were often recruited either after a first or possibly master's degree, most recruits to biotechnology had had some relevant postgraduate research experience and were typically in their mid to late 20s or early 30s. Apart from new companies or centres starting up, the numbers recruited rarely exceeded ten vacancies in a year in any one company. Wastage rates were also generally low. The only significant shortage of suitably experienced personnel was in fermentation technology, particularly microbial physiologists, biochemical engineers and bioscientists with research and development experience of plant and tissue cell culture. There was also a shortage, as in many other technologies, of people who could combine technical and managerial expertise.

One particular concern of the past few years has been the potential scale of the brain drain (see *Nature* 308, 572; 1984). The study estimated that about 250 UK nationals had gone abroad since the mid-1970s, representing perhaps 13 per cent of the number currently employed in the United Kingdom. The peak flow was about 30 per annum in 1981 and 1982. UK nationals were found working in 13 countries; the main destinations were the United States (46 per cent), followed by Switzerland (16 per cent) and then Canada,

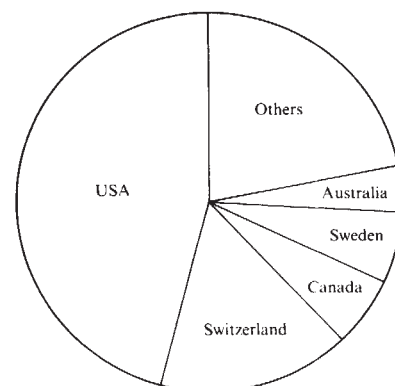


Fig.2 UK biotechnologists taking positions overseas.

Sweden and Australia (Fig.2). The largest loss was from higher education institutions and the main flow was to commercial organizations. More than 65 different overseas organizations were known to have recruited UK nationals. One recruited at least 16 UK nationals, four others took four or more each. One in three of those who had gone overseas had changed jobs since leaving the United Kingdom, and one in eight had also changed countries.

Typically the leavers were aged 26 to 30, although one in three was over 30. Nearly one in three went to "senior" jobs overseas, at professorial or departmental head level. They reported that the main reason for leaving was not salary but the lack of "suitable" opportunities and jobs in the United Kingdom. Most of them did not expect to return to the United Kingdom because they would be unwilling to take a reduced salary and standard of living. Thus while salary is not a motivation in leaving, it is a barrier to returning. While the flow overseas included a number of people who had left senior appointments in the United Kingdom and were unlikely to return, some were young postdocs moving overseas for experience, part of the normal international interchange of scientific personnel. While the outflow had no significant impact on individual organizations, such losses being only one part of their wastage to other employers, the flow was regarded as a lost opportunity and weakening of the United Kingdom's scientific base. The future flows, in either direction, will depend on job opportunities in the United Kingdom and salary differentials. □

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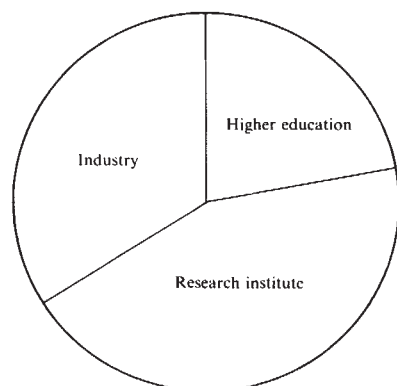


Fig.1 Distribution of biotechnologists in UK institutions.